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STUDIES ON THE PHYSIOLOGY OF SLEEP

III. The Effect of Muscular Activity, Rest and Sleep on the Urinary Excretion of Phosphorus

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For a long time it was believed that mental activity had an influence on phosphorus metabolism, and numerous experiments were undertaken to determine the effect of intellectual work on the elimination of phosphorus in the urine. The various investigators were especially interested in determining the effect of sleep on the excretion of phosphorus in the urine, since sleep is a period of minimal activity. Thus, to the exclusion of other constituents of the urine, there were accumulated in the literature a large number of data on phosphorus in the night and day urines. All workers partitioned their 24-hour samples into two equal 12-hour periods. Of the eleven authors whose figures were quoted by Piéron in his book on sleep (1913), four found that more phosphorus was excreted at night, four held that more of it was excreted in the daytime, and three could detect no difference in the quantity of phosphorus in the day and night urines. Campbell and Webster (1921) have given convincing evidence of an increase in the excretion of phosphorus during the night, but these investigators, too, partitioned their samples into two equal periods, so that their night urine contained not only the phosphorus excreted during 8 hours of sleep, but also that excreted in 4 hours of wakefulness. In a previous paper the writer (1923) separated the 24-hour urine into an 8-hour period of sleep and a 16-hour period of wakefulness, and the results confirmed the observation of Campbell and Webster concerning the increase in the excretion of phosphorus during sleep. But whereas a diminished excretion of phosphorus could be understood on the basis of the notion as regards the effect of mental activity on the metabolism of phosphorus, a definite

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increase in the elimination of phosphorus called for a further investigation of the question of urinary phosphorus.

Aside from the decrease in mental activity during sleep, there is also present a more or less complete relaxation of the body musculature. We decided to investigate these two phases separately. In one instance we followed the phosphorus elimination in sleep and wakefulness for many weeks as a normal control, and after this, during a period of presumably decreased mental activity resulting from the daily administration of comparatively large doses of potassium bromide. To study the effect of muscular relaxation we analyzed the urine secreted during short periods of strenuous muscular work, and that secreted during like periods before and after the exercise.

The literature on the effect of work on the elimination of phosphorus is even more voluminous than that on the influence of sleep, and the results are just as contradictory. As far back as 1859 Speck found that the excretion of P_2O_3 during rest was 1820 mgm. per 24 hours, but that this was increased to 2680 mgm. as a result of muscular work. Pettenkofer and Voit (1866), also working on man, obtained 4.11 grams of H_3PO_4 in the urine secreted in 24 hours of rest, as against 4.19 grams for a like period when some work had been performed, thus showing that there was no appreciable difference that could be ascribed to work. A nitrogen-free diet combined with rest resulted in a diminished excretion of phosphoric acid, 3.15 grams per day, and during fasting, when some work was done, the excretion of phosphoric acid fell further to 2.95 grams. Engelmann (1871) found an increase in the 24-hour output of phosphoric acid as a result of work. He employed three subjects and in each determined the average excretion of H_3PO_4 in three-day tests. The corresponding figures for work and rest days in the three individuals were 3.505 and 3.521, 3.249 and 2.819, and 3.160 and 2.711. Thus in two subjects distinctly more phosphoric acid was excreted during work days, while in the third slightly more during rest. Oertel (1898), on the other hand, reported a decrease in the excretion of phosphorus on working days, his subjects being intellectual workers. Penzoldt and Fleischer (1882) analyzed the urine of a bitch and found the daily excretion of H_3PO_4 to be about 750 mgm., slightly more during the night than during the day. When one day the bitch was made to work for two hours, the day urine contained only 20 mgm. of H_3PO_4 instead of the usual 370, and the night urine 710 mgm. instead of 400. This difference is so great, that in view of the other data in the literature these results cannot possibly be correct, but somehow many writers quoted these figures as indicating a decrease in the excretion of phosphorus as a result of work. Penzoldt and Fleischer did not repeat the experiment. Kaup (1902) using human subjects found a decrease in the 24-hour excretion of P_2O_5 on working days. His figures are interesting in another con-

nection, as will be shown later. Recently Embden, Schmitz andMeincke (1921) reported that sodium phosphate given by mouth resulted in an increased ability to perform work in their subjects (soldiers). In the same subjects Embden and Grafe (1921) detected a distinct, though not always consistent, increase in the 24-hour excretion of phosphoric acid on days of work. They published detailed figures of the phosphoric acid content of

TABLE 1

Total phosphorus and total acidity in the urines secreted during sleep and during wakefulness

A. Total phosphorus in milligram of P

DATES OF TEST	DURATION	HOURS ASLEEP	HOURS AWAKE	VOLUME OF URINE		P IN 100 CC.		TOTAL P		P PER HOUR	
				Asleep	Awake	Asleep	Awake	Asleep	Awake	Asleep	Awake
	hours			*cc.*	*cc.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
January 26–30	96	32.47	63.53	1250	3355	103	65	1294	2194	39.7	34.6
February 2– 6	96	33.42	62.58	1545	2605	123	110	1911	2889	57.2	46.2
February 9–13	96	32.45	63.55	2020	2460	82	102	1655	2529	51.0	40.0
February 17–19	48	16.63	30.74	750	1220	87	97	726	1061	43.6	34.5
February 23–27	96	35.98	60.02	2225	2665	77	82	1703	2192	47.3	36.5
March 2–6	96	34.00	62.00	1100	2335	181	124	1988	2897	58.5	46.7
April 13–17	96	35.85	60.15	2680	3235	67	77	1807	2497	50.4	41.5
April 20–24	96	32.35	63.65	2885	3570	47	62	1329	2233	41.0	35.1

B. Total acidity in cubic centimeter of N/10 acid

DATES OF TEST	DURATION	HOURS ASLEEP	HOURS AWAKE	VOLUME OF URINE		N/10 ACID IN 100 CC.		TOTAL N/10 ACID		N/10 ACID PER HOUR	
				Asleep	Awake	Asleep	Awake	Asleep	Awake	Asleep	Awake
	hours			*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
February 23–27	96	35.98	60.02	2225	2665	31.6	35.0	703	833	19.5	13.9
March 2–6	96	34.00	62.00	1100	2335	65.0	35.0	715	817	21.0	13.2
March 9–13	96	32.68	63.38	2320	3355	25.0	26.4	580	886	17.7	14.0
March 16–19	72	25.60	46.40	1205	2045	25.6	28.0	429	573	16.8	12.4
April 13–17	96	35.85	60.15	2680	3235	21.0	27.5	563	890	15.7	14.8
April 20–24	96	32.35	63.65	2885	3570	19.0	23.0	551	828	17.0	13.0

two-hourly samples of urine throughout the day, and we shall make use of them both for the analysis of their findings, and for substantiating certain conclusions drawn from our own experiments.

METHODS AND RESULTS. For the study of the influence of mental activity on the output of posphorus we used a subject who was making metabolic studies on himself and had to collect urine for analysis. He ate simple foods whose composition he knew, and he weighed them accurately. The method of partitioning the 24-hour sample into two periods of 8 and 16

hours, carried out in the previous work, was not followed in this case. The subject collected separately the urine he excreted from the time he turned off the light at night till the moment he woke up in the morning, and this we shall call the sleep-urine. The urine collected between that moment and the time he went to bed at night constituted the wakefulness-urine. He was not required to go to bed and to get up at definite hours, but was allowed to sleep as much as he cared, which was between 8 and 9 hours. The period of collection was generally 96 hours, from Monday morning to Friday morning. The four sleep-urine samples were mixed together, and phosphorus and acidity determined. The same was done for the combined wakefulness-urine. For the total acidity determination we used the Folin titration method and for the total phosphorus the Neumann-Pemberton method. All samples were analyzed twice.

The results obtained are given in table 1. In the first six periods the excretion of phosphorus during sleep was consistently greater than during wakefulness, the increase varying from 15 to 29 per cent. It is interesting to note that the increased excretion of phosphorus during sleep is entirely independent of the volume of urine secreted. Thus in the four-day period, February 23 to 27, large quantities of water having been drunk in the evening, 2225 cc. of urine were secreted during 36 hours of sleep, and 2665 cc. in 60 hours of wakefulness. The corresponding amounts of phosphorus excreted were 1702 mgm. and 2192 mgm., 47.3 mgm. having been excreted per hour during sleep and 36.5 mgm. during the waking period. The following week, in the four-day period of March 2 to 6, only 1100 cc. of urine were secreted during 34 hours of sleep, and 2335 cc., more than twice as much, in 62 hours of wakefulness. Yet in this case, too, much more phosphorus was excreted in sleep than in wakefulness, the corresponding figures being 58.5 and 46.7 mgm. per hour. The figures for the total acidity expressed in cubic centimeters of N/10 acid follow closely those for total phosphorus. After this relationship had been established, the subject was given from 2.7 to 3 grams of potassium bromide daily, in three equal doses. At the end of one month an equilibrium was supposed to have been established, and the subject had about 3 grams of KBr in his system at all times. The effect of the bromide was not always depressive, as occasionally the subject reported a certain degree of hyperexcitability. But as a whole the bromide acted as a sedative, the subject was often sleepy and at times felt "dopy." The urine collected during the two four-day periods following, the KBr medication being continued, showed the same augmentation in the excretion of phosphorus and acid during sleep as in the previous control periods. It thus seems probable that the excretion of phosphate is independent of intellectual activity.

We then decided to investigate the effect of work and rest on the elimination of phosphorus. As pointed out, the results obtained by previous

workers were highly contradictory. We determined in our subjects the quantity of phosphorus excreted in each of three successive two-hour periods, with the subject remaining in bed during the first and third periods and working during the second period. On another day the subject

TABLE 2

The effect of exercise upon the excretion of total phosphorus and total acids in the urine

	CONTROL			TEST		
	6–8 rest	8–10 rest	10–12 rest	6–8 rest	8–10 work	10–12 rest
Subject II:						
Volume of urine, cc.	335	485	290	292	94	210
P, mgm.	56.5	34.3	28.4	60.0	26.9	38.2
N/10 acid, cc.	16.8	11.6	9.9	30.4	22.2	9.2
Volume of urine, cc.	352	452	376	262	73	102
P, mgm.	50.0	41.9	35.9	62.7	20.7	42.2
N/10 acid, cc.	22.5	13.1	9.0	16.2	16.1	8.4
Subject V						
Volume of urine, cc.	105	290	350	364	122	208
P, mgm.	69.8	53.4	53.3	40.7	23.4	37.4
N/10 acid, cc.	15.9	5.5	8.0	12.4	6.1	9.7

	CONTROL			TEST		
	6–7½ rest	7½–9 rest	9–10½ rest	6–7½ rest	7½–9 work	9–10½ rest
Subject III:						
Volume of urine, cc.	176	226	262	210	392	278
P, mgm.	26.1	21.3	16.8	35.7	19.4	27.6
N/10 acid, cc.	8.6	6.3	5.2	10.9	6.7	4.4
Subject IV:						
Volume of urine, cc.	300	385	268	182	70	89
P, mgm.	28.3	34.0	33.7	39.9	36.1	40.4
N/10 acid, cc.	9.7	7.5	6.2	21.7	7.8	16.4

	CONTROL					TEST				
	6–7 rest	7–8 rest	8–9 rest	9–10 rest	10–11 rest	6–7 rest	7–8 work	8–9 rest	9–10 work	10–11 rest
Subject II:										
Volume of urine, cc.	143	322	286	136	166	102	71	92	54	154
P, mgm.	27.5	25.5	16.0	12.6	14.2	28.8	14.5	17.1	13.8	21.1
N/10 acid, cc.	8.0	3.3	2.4	1.7	2.0					

remained in bed during the entire six hours, and by comparing the two curves of phosphorus excretion any difference due to work could be easily detected. The subjects were all in the post-absorptive state; they ate for the last time at 6 p.m. on the eve of the test. The test itself lasted from 6

a.m. to 12 m. The form of exercise chosen was such as to suit all subjects (who were physically untrained); namely, brisk walking, so brisk as to be distinctly unpleasant, and it generally became painful toward the end of the two-hour period. The distance covered in two hours was 8.5 miles. Two hundred cubic centimeters of water were taken by each subject at the beginning of each two-hour period. Four male subjects, students at the University, were employed for these tests. The figures for phosphorus and acidity are shown in table 2. It will be seen that exercise affected markedly the curve of phosphorus excretion. On rest days the amount excreted in the third period was always less than in the second, whereas

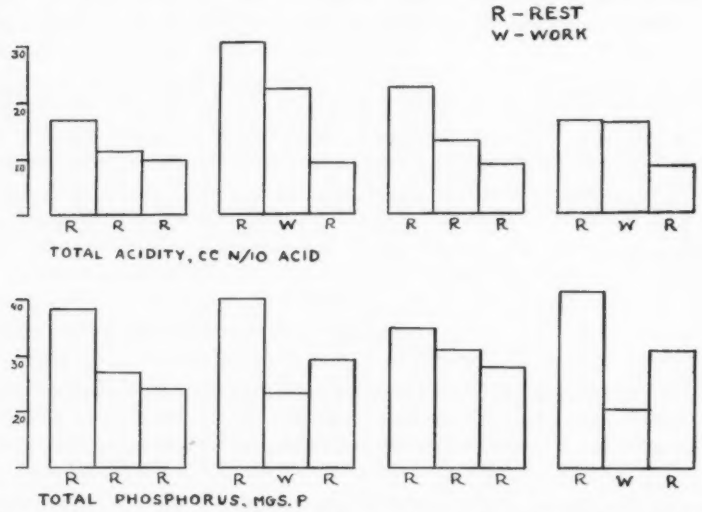

Fig. 1. The rate of excretion of phosphorus and acid during four six-hour periods. Time, 6 a.m. to 12 m. Subject II. *R*—two-hour period of rest, *W*—two-hour period of work.

on work days the reverse was true. Apparently some of the phosphorus which in the ordinary course of catabolism would be excreted during the second period was *retained* in the body because of the work, but the amount retained was promptly excreted within the two hours following, when the subject was allowed to lie down and rest. This was especially clearly shown in a test where the urine was collected hourly, and 100 cc. of water were drunk before each period. The subject rested during the first, third, and fifth hours, and walked during the second and fourth hours. The phosphorus excretion shows a zigzag curve quite distinct from the smooth curve obtained on another day when the subject rested all five hours in bed. Incidentally the total amount of phosphorus excreted in five

TABLE 3

The rate of excretion of total phosphorus and of total acidity in fasting subjects during the day

	5½–7	7–8½	8½–10	10–11½	11½–1	1–2½	2½–4	4–5½	5½–11½	11½–5½
Subject I. Two tests, each on first day of fasting:										
Volume of urine, cc.	185	370	85	50	55	45	35	30	690	165
P, mgm.	58.3	37.6	21.7	17.4	36.9	50.8	58.8	55.4	135	202
Volume of urine, cc.	280	325	90	150	135	135	145	125	765	540
P, mgm.	48.1	30.5	20.2	19.5	48.4	70.5	71.8	64.2	118.3	254.9

	6–8	8–10	10–12	12–2	2–4	4–6	6–12	12–6	REMARKS
Subject II:									
Volume of urine, cc.	92	121	74	45	37	34	287	116	First day of fasting
P, mgm.	39.4	22.8	19.0	26.7	28.0	31.8	81.2	86.5	
N/10 acid, cc.	15.3	8.0	17.5	20.0	20.9	20.4	40.8	61.3	
Volume of urine, cc.	256	388	170	170	182	309	814	661	First day of fasting
P, mgm.	40.9	25.5	19.4	30.4	31.6	40.5	85.8	102.3	
N/10 acid, cc.	13.8	6.4	6.7	19.4	19.7	22.0	26.9	61.1	
Volume of urine, cc.	45	70	144	76	77	72	259	225	Following day, second day of fasting
P, mgm.	46.4	41.1	35.9	52.0	62.2	73.4	123.4	187.6	
N/10 acid, cc.	23.9	22.4	20.6	28.0	34.5	38.9	66.9	91.4	
Subject VI:									
Volume of urine, cc.	51	134	50	44	46	46	235	136	Eighth day of fasting
P, mgm.	50.9	52.4	51.6	63.5	67.2	66.3	154.9	197.0	
N/10 acid, cc.	27.3	35.8	34.0	31.2	30.0	32.5	97.1	93.7	
Volume of urine, cc.	82	106	46	125	170	134	234	429	Fourteenth day of fasting
P, mgm.	34.6	31.4	35.4	47.7	49.6	46.1	101.4	143.4	
N/10 acid, cc.	12.3	14.0	18.0	35.0	27.9	26.0	44.3	88.9	
Subject VII:									
Volume of urine, cc.	70	60	58	58	66	81	188	205	Sixteenth day of fasting
P, mgm.	3.3	12.5	21.1	37.1	24.7	58.7	36.9	120.5	
N/10 acid, cc.	2.9	6.6	8.5	15.7	9.1	22.8	18.0	47.6	

hours on each of these days was exactly the same, 95 mgm. The curve for the total acidity shows a more stable rhythm than the phosphorus curve, when the rhythm is present, which is not always the case. There is generally less acid excreted in each succeeding period during the morning, but this is not affected by work.

In examining the figures for phosphorus obtained in the control test we were struck by the fact that in all but one case there was a gradual decrease in the quantity of phosphorus excreted per hour. We decided to see whether this continued in the afternoon. In order to avoid the possible

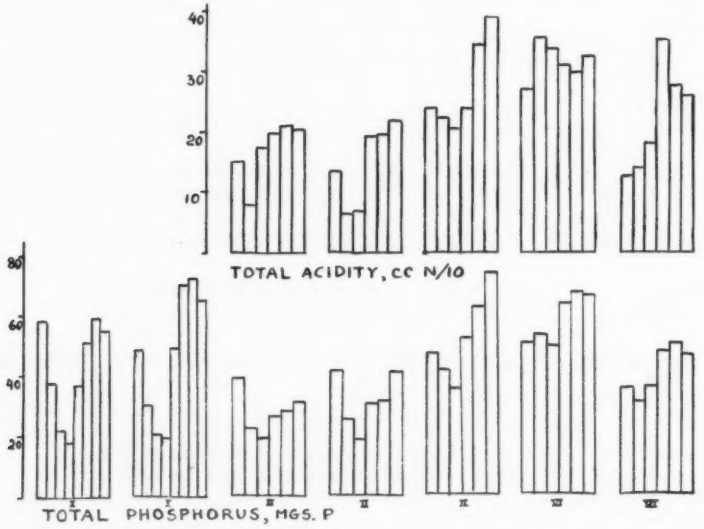

Fig. 2. The rate of excretion of phosphorus and acid during the day, 6 a.m. to 6 p.m. Four subjects. The first four charts for subjects I and II were taken on the first day of fasting. The fifth is on the second day of fasting (subject II). The last two are of the 7th and 13th days of fasting.

effect of digestion, we decided to let the subjects fast during 24 hours, beginning at 6 p.m., the test itself running from 6 a.m. to 6 p.m. on the following day. Four subjects were employed. In the case of subject I, the urine was collected in eight 90-minute periods. There was a definite curve for the phosphorus excretion which reached its lowest point at noon and its peak toward the evening. In the other cases urine was collected in six two-hour periods (200 cc. of water at the beginning of each period). Subject II showed a curve similar to that of subject I. Once he fasted for 48 hours, so that the phosphorus excretion could be followed for two consecu-

tive days. On the second day of fasting much more phosphorus was excreted than on the first, but the curve remained the same. The greater excretion of phosphorus might have been due to greater destruction of protein. Subject VI was studied on the 7th and 13th days of fasting, and subject VII on the 16th. These subjects show no definite curve of phosphorus excretion. What is shown by each of the four subjects in the seven tests is a much greater output of phosphorus from 12 m. to 6 p.m. than from 6 a.m. to 12 m. As in the case of the work and rest tests the figures for the total acidity do not always follow very closely those for phosphorus, but on the whole there seems to be a definite direct relationship between the two. This is especially well shown in the figures for the second day of fasting in subject II and the 16th day of fasting in subject VII.

DISCUSSION AND CONCLUSIONS. The results obtained in the case of subject I show unmistakably that the urine actually secreted during sleep has a much greater content of phosphorus and acid than that secreted in the waking state, confirming the results of previous less rigidly controlled

TABLE 4

Rearranged data of Kaup (1902), showing an increase in the excretion of urinary P during sleep

DAY	TOTAL P_2O_5	7 a.m.–9 p.m.	9 p.m.–7 a.m.	MILLIGRAMS PER HOUR, "DAY"	MILLIGRAMS PER HOUR, "NIGHT"	REMARKS
	mgm.	*mgm.*	*mgm.*			
I	2935	1335	1600	95	160	
II	2580	1350	1230	95	123	
III	1860	760	1100	54	110	Worked during day
IV	2805	1200	1605	86	160	

tests in this and other laboratories. The administration of potassium bromide failed to disturb this relationship, indicating that mental activity hardly played a part in determining this lesser excretion of phosphorus during wakefulness. Kaup's figures (1902) properly arranged show a definite and very considerable increase in the excretion of phosphorus during sleep. In the 14-hour period from 7 a.m. to 9 p.m. the hourly excretion of P_2O_5 varied from 54 to 95 mgm., and in the 10-hour period from 9 p.m. to 7 a.m., the greater part of which was presumably spent in sleep, the hourly values vary from 110 to 160 mgm. (table 4).

That there is a parallelism between the rate of excretion of phosphorus and that of acid had been noticed by all observers who determined simultaneously these two constituents of the urine. Among these were Fiske (1921), Sokhey and Allan (1924), Bazett and his co-workers (1924). The question has often been asked whether the phosphate excretion followed that of the acid, or whether the acidity of the urine was determined by the amount of phosphorus excreted. No one suggested that they might be

independent of each other, since they do seem to run parallel. Campbell and Webster take up the theory according to which "sleep is said to be due to the accumulation of acid products in cells, these acids being excreted more slowly than they are formed during the day, and thus collecting in the cells with a resulting diminution of activity of the cells, particularly in the brain cortex." They suggest that "the cells of the body may not excrete certain fixed acids into the blood until certain amounts are formed in each cell. When this is the case these acids are excreted into the blood and fatigue or sleep is produced." They conclude that "the phosphate tide at night is considered to be due to the increased acidity of the urine. It did not appear to be connected with muscle or nerve metabolism in particular." These two conclusions appear to be incorrect. In the first place, the excretion of phosphorus is not always parallel to that of acid. As is shown by the results of the exercise tests, work may entirely upset the normal curve for phosphorus excretion without affecting the curve for total acidity. In the second place, phosphorus excretion *is* connected with muscle metabolism. The normal curve for phosphorus excretion shows a gradual decrease during the morning hours. But if exercise is taken, there is a marked decrease in the phosphorus output, as compared with normal, during the exercise, and a corresponding rise in the period immediately following the exercise. It seems probable that during the exercise some phosphorus is taken up by the muscle, and therefore not all the phosphorus catabolized, is excreted by the kidneys. After the work has been discontinued the phosphorus is given up by the muscles and added to the phosphorus catabolized, and the output of phosphorus is greater than it would have been normally for this period. The possible decreased circulation through the kidney has been held responsible for the decrease in the excretion of phosphorus by some investigators, but since the other constituents of the urine are not affected by work in the same way, this supposition is evidently wrong.

Sokhey and Allan (1924) have found that the injection of insulin or administration of sugar cause a decrease in the inorganic phosphorus in the urine for about 6 hours, and an increase in the 6 hours following. They see an evidence of the "existence of an intimate relationship between the metabolism of carbohydrate and phosphoric acid. It would appear that after the injection of carbohydrate, or after the administration of insulin when change in glucose is taking place, there is a demand for phosphate to form some compound with a carbohydrate product. This compound exists only temporarily and when it breaks up, the phosphoric acid is released, and the excretion therefore increased." Thus the total excretion of phosphorus at any one period is the difference between the amount catabolized by the cells of the body and thrown into the blood stream and the quantity taken up by the tissues in connection with carbohydrate

metabolism. Muscular work may be looked upon as an example of increased carbohydrate metabolism, and it involves a temporary binding

TABLE 5

Rearranged data of Embden and Grafe (1921), showing the increase in the excretion of urinary P during the afternoon

DATE OF TEST	SUBJECT Ma			SUBJECT Schu		
	6a.m.–12 m. mgm. H_3PO_4	12 m.–6 p.m. mgm. H_3PO_4	Remarks	6a.m.–12 m. mgm. H_3PO_4	12 m.–6 p.m. mgm. H_3PO_4	Remarks
June 3	624	1261	Rest	612	1346	Rest
4	713	1044	Rest	868	1148	Rest
5	860	1304	Work	808	1124	Work
6	876	1316	Work	860	1068	Work
7	836	1336	Work	940	1108	Work
8	872	1248	Work	1084	*1024*	Work
9	660	1228	Rest	896	1356	Rest
10	752	1224	Rest, partly in bed	1000	1276	Rest
13	528	1156	Rest, partly in bed	688	872	Rest
14	496	1148	Complete rest in bed	807	984	Rest in bed
15	508	984	Complete rest in bed	744	1092	Rest in bed
16	788	1424	Hard work	992	1312	Hard work
17	1184	1392	Hard work	1208	*1120*	Hard work
18	679	1296	Complete rest in bed	836	1284	Rest in bed
19	700	1052	Complete rest in bed	936	1224	Rest in bed
20	544	1004	Complete rest in bed	908	1246	Rest in bed
July 9	376	738	Rest	544	856	Rest
10	316	828	Rest in bed	721	1080	Rest in bed
11	612	1352	Work	928	1144	Work
12	972	1524	Work	1100	1236	Work
13	1012	1424	Work	1024	1204	Work
14	714	1252	Rest in bed	1025	1442	Rest in bed
15	716	1092	Rest in bed	944	1208	Rest in bed
17	520	997	Rest	922	1111	Rest
18	539	1184	Rest in bed	832	1172	Rest in bed
19	596	1112	Rest in bed	744	1156	Rest in bed
20	692	1104	Rest in bed	800	1068	Rest in bed
21	673	1080	Rest in bed	920	1212	Rest in bed
22	872	1344	Work	1004	1250	Work
23	1216	1488	Work	1128	*1044*	Work

of some phosphorus by the muscle tissue and the giving up of this phosphorus in a very short time, one or more hours. If the work be continued

for many hours, as in the experiments of Embden and Grafe (1921), the phosphorus bound in the first hours of work may be excreted during later hours of work, thus giving an impression of an increased excretion of phosphorus *during* work, which is confusing and misleading. This may explain the contradictory results obtained by earlier workers, some maintaining that muscular work causes an increase in the excretion of phosphorus, others that it causes a decrease.

That there is an actual accumulation of inorganic phosphorus in muscle as a result of work was demonstrated by Macleod (1899) who determined the phosphorus content of rested and exercised muscle tissue of dogs. The average amount of total phosphorus in 100 grams of dried muscle tissue was 376 mgm., but this was increased to 444 mgm. in muscles of dogs exercised to the point of fatigue.

When the rate of phosphorus excretion was followed throughout the day, there was more of it excreted in the afternoon than in the morning. Similar results were obtained by Embden and Grafe (1921) who apparently overlooked this phenomenon. It appears very clearly in the rearranged figures of Embden and Grafe given in table 5. Fiske (1921) obtained an increase during the afternoon, and he thought that either there was actually less phosphorus catabolized in the morning, or there was a retention of phosphorus which was "released" later in the afternoon. Sokhey and Allan noticed that their dogs excreted more phosphorus in the afternoon than in the morning, whether they were fed or fasting. From the rearranged data of Embden and Grafe one can see that only in three cases out of sixty was there more phosphorus (and then only slightly more) excreted in the morning than in the afternoon, but this always happened after one or more days of work, indicating that the extra amount of phosphorus excreted was probably that retained for a slightly longer period as a result of work. Sokhey and Allan found that insulin caused an increase of from 25 to 35 per cent in the daily output of phosphorus, but that the increase sometimes appeared partially on the following day. The length of time, then, during which the phosphorus taken up as a result of carbohydrate metabolism is retained, is variable; it may be given up in one hour, as shown in our short period exercise tests, or only after many hours.

From the foregoing it seems probable that the increase in the excretion of phosphorus during sleep is due to more complete muscular relaxation and to a corresponding decrease in carbohydrate metabolism. One will recall that there is a marked lowering of the respiratory quotient during sleep which also points to a decrease in carbohydrate metabolism. The diminution in the excretion of phosphorus in the morning hours may be due to increased tonus of the muscles and the withdrawing of phosphorus from circulation, and the increase in the phosphorus output in the afternoon to a gradual decrease in the tonus of the muscles and to the release

of the phosphorus taken up in the morning. As we have seen, this holds true whether the individual is fed or starving for many days, whether he works or rests, or remains in bed. Those individuals who show a definite smooth curve of phosphorus excretion, with the lowest point about noontime, possibly possess a gradually increasing muscular tonus (efficiency?) during the early part of the day and a gradual ebbing of the tonus in the afternoon. As regards the connection between sleep and the increased excretion of phosphorus which accompanies it, it seems that the latter is in no way related to the mechanism of sleep as such, but is merely a *concomitant* of sleep, depending directly upon complete muscular relaxation.

SUMMARY

1. More phosphorus is excreted in the urine per hour during sleep than during wakefulness, independently of the volume of urine secreted. The same applies to total acidity. Three grams of potassium bromide daily for a long period has no effect on this relation.

2. During short periods of muscular work (1 to 2 hours) phosphorus is retained in the body, and it is excreted shortly after the exercise has been discontinued.

3. There is an increase in the total acid and total phosphorus excreted per hour in the afternoon as compared with the morning. In some individuals the rate of excretion shows a regular curve, with its lowest point about noon.

4. It is suggested that the increase in the excretion of phosphorus during sleep is due to the more complete muscular relaxation in that state.

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STUDIES OF THE PHYSIOLOGY OF THE LIVER

XII. Muscle Glycogen Following Total Removal of the Liver

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The most important immediate effect of complete removal of the liver from animals is the decrease in the amount of sugar in the blood. We have shown with different species of animals that the duration of life following hepatectomy depends directly on the rate of disappearance of the sugar from the blood (Mann and Magath, 1921). This is also true in the individuals of any species we have studied. The death of the dehepatized animal is associated with a hypoglycemia and always occurs when the sugar of the blood has decreased to a definitely small amount. Administration of glucose to dehepatized dogs immediately relieves the symptoms of hypoglycemia and enables the animals to survive many hours longer (Mann and Magath, 1922).

The glucose deficiency produced by excision of the liver is the direct result of the elimination of two of the body's sources of glucose. The mechanical removal of the available glucose and glycogen stored in the liver is undoubtedly of immediate importance to the organism. The absence of the liver also prevents the formation of new glucose from the proteins of the body, as evidenced by the complete cessation of the formation of urea and by the accumulation of amino-acids in the blood and tissues (Bollman, Mann and Magath, 1924). Amino-acids form neither glucose nor urea in the absence of the liver.

However, a considerable amount of glycogen is present in the muscles so that the amount of glycogen in the dehepatized animal is as great as, or greater than, the amount removed with the liver (Maignon, 1908). This muscle glycogen serves as a source of energy for muscular activity and perhaps furnishes much of the energy necessary for maintenance of body temperature (Myerhof, 1924). In the absence of hepatogenic glucose it would seem that this muscle glycogen might be converted into glucose as is liver glycogen in the normal animal to maintain the normal level of sugar in the blood and to produce hyperglycemia in times of additional

stress. The hypoglycemia of dehepatization shows that the muscle glycogen does not maintain this important function of the liver. The changes in the glycogen content of the muscles not affected by the presence of the liver seem to have an important bearing on both the physiology of muscle and the physiology of the liver in the function of regulating the sugar concentration in the blood.

Aron (1922) has made certain interesting and suggestive investigations on the distribution of glycogen in the embryo. He found that in early life glycogen is distributed to all the primitive tissues. Later, with the development of the islands of Langerhans, the glycogen becomes much more concentrated in the liver, and at birth the liver is rich in glycogen while the other tissues contain very much smaller amounts.

De Filippi (1907) showed that there is a lowered tolerance for carbohydrates subsequent to making Eck fistulas in dogs. He found that in such animals the muscles contained an increased quantity of glycogen, characteristic of over-nutrition, and a diminished content of hepatogenic glycogen, characteristic of inanition. He concluded that the muscles can form glycogen independently of the liver and that the liver is neither specific nor indispensable for carbohydrate metabolism. These findings have been confirmed by Jacobson (1920) who found, however, that the glucose tolerance is only slightly modified by Eck's fistula. He also concluded that the liver is not essential for the metabolism of glucose, and that the muscles apparently perform well the functions of glycogenesis and glycogenolysis after the circulation to the liver has been altered. He further showed that glucose tolerance is augmented in such animals subsequent to removal of the posterior lobe of the hypophysis, which indicates an increased glycogenic capacity of the muscles in this condition. Moehlig and Ainslee (1925) infer that the posterior pituitary extract has an antagonistic action to that of insulin because glycogenolysis by the muscle is increased under the influence of the pituitary extract.

Külz (1881) injected glucose subcutaneously into dehepatized frogs and found a slight increase in the glycogen content of the muscle. Torti (1912) also reported similar findings in dehepatized frogs, even without the injection of glucose. Sachs (1899) showed that the tolerance for dextrose, galactose and arabinose is not lowered by extirpation of the liver in frogs, but that a marked decrease in the tolerance for levulose occurs.

Laves (1887) removed the livers from ducks and geese and found a marked diminution in the glycogen content of the muscles. He was unable to demonstrate any increase in the muscle glycogen even though he fed his dehepatized animals from 20 to 30 grams of grape sugar. Kausch (1897) injected glucose subcutaneously into dehepatized ducks and geese. He found that the blood sugar and muscle glycogen decreased, and no evidence of glycogen formation in the dehepatized animal was obtained.

In perfusion experiments with surviving muscle from the dog, Külz (1881) found that glycogen could be formed from the common monosaccharids and disaccharids by the isolated muscle. Hatcher and Wolf (1907), in repeating these experiments, found that glucose perfusion gave rise to an increase in the glycogen content of the perfused muscle, but that other sugars were ineffective in the production of glycogen.

METHODS. Since the method of extirpation of the liver has already been detailed (Mann, 1921), only a brief description will be given here. The liver is removed in three stages. All operations are performed under ether anesthesia with aseptic technic.

The object of the first operation is to establish a reverse Eck fistula, that is, lateral anastomosis of the portal vein and the vena cava and ligation of the latter on the cephalic side of the stoma. At first a considerable portion of the blood from the posterior portion of the body passes through the liver, but since the capillaries of the liver offer more resistance to the flow of blood than is necessary for the development of collaterals through the azygos and internal mammary veins, most of the blood soon passes by way of the latter channels.

The second operation consists in the ligation of the portal vein at its entrance to the liver. This causes all the blood from the viscera and hind legs to return by way of the collateral vessels, the azygos and the internal mammary veins.

At the third operation the entire liver is extirpated intact, along with the portion of the vena cava which it surrounds.

Two specimens of muscle, of approximately 10 grams each, were obtained at the time of operation immediately following removal of the liver. Recovery from anesthesia and from the immediate effects of the operation is rapid. Within one hour after operation, the animal is to all appearances normal; it walks around, responds to call, exhibits the usual interest in its surroundings, and drinks water. After a period of from two to eight hours, it develops the characteristic symptoms of hypoglycemia. Muscular weakness is the first symptom noted. The animal cannot hold itself erect, or avoid swaying when walking, and prefers to lie down. The muscular weakness increases quickly and in a short time the animal is unable to move any of its muscles except those necessary for respiration. At the same time the reflexes decrease and they are subsequently lost. Usually, within an hour after evidence of muscular weakness is first noted, the animal lies quietly breathing, with muscles relaxed and flaccid. After a variable period, usually not more than one hour, there is a rather sudden return and exaggeration of the reflexes. Muscular twitchings appear, at first involving separate muscles or small groups of muscles, then general convulsions occur and the animal dies.

The second specimens of muscle for determination of glycogen were ob-

tained immediately after the onset of the muscular twitchings. Glucose was then administered intravenously with the usual prompt return of the animal to normal. A hyperglycemia was then maintained by the administration of glucose for a period of several hours during which the animal again appeared normal.

The third specimens of muscle for determination of glycogen were obtained immediately after the death of the animal. In view of the possible variation in the glycogen content of the different muscles and any possible variation due to local trauma, nerve injury and so forth, the sites of choice were varied in each experiment. The muscles used were the vastus medialis, vastus lateralis, semimembranosus, gracilis and rectus femoris. Two specimens of approximately 10 grams each were obtained from a fresh site for each determination.

Blood specimens for determination of sugar were obtained at varying intervals in a dry syringe from the jugular vein and mixed with powdered oxalate in dry tubes.

The determinations of blood sugar were made on the whole blood by the method of Folin (Folin and Wu, 1920), and in a few instances by the Benedict (1918) modification of the Lewis-Benedict method.

Glycogen was determined by the method of Pflüger (1909) with some modifications which are described. Small stoppered flasks containing 10 cc. of 60 per cent sodium hydrate were tared, and the muscle specimens placed in them immediately after removal. The flasks were immediately weighed and the increase taken as the weight of the muscle. Each flask was then placed in a boiling water bath and the amount of 60 per cent sodium hydrate added (if necessary) to make 1 cc. of hydrate for each gram of muscle contained. In routine this was completed within three minutes after excision of the muscle. The flask was heated on the boiling water bath, with intermittent shaking, for two hours. The contents, with the muscle now completely digested, were emptied into a beaker and the flask washed with an equal volume of water. To the cooled mixture, twice the volume of 95 per cent alcohol was added and the contents well mixed. The beaker was covered and allowed to stand overnight.

The mixture was then filtered and the precipitate washed with 66 per cent alcohol until the washings were colorless. Washing was then repeated with 95 per cent alcohol and absolute alcohol, then with alcohol and ether, and finally with ether. The precipitate was then transferred to a small flask and the filter paper washed repeatedly with boiling water until 40 cc. had been used. This was then neutralized with concentrated hydrochloric acid, and 2 cc. concentrated hydrochloric acid (specific gravity 1.19) added. The flasks were then placed in a boiling water bath for three hours for hydrolysis of the glycogen, then cooled and neutralized with concentrated sodium hydroxid. This solution was then made

up to 100 cc. with water in a volumetric flask, filtered, and the glucose determined as the filtrate in Folin's blood sugar method. If the colorimetric readings were below 15 (standard 20) the proper dilution was made and the sugar determination repeated.

For calculation of glycogen the factor 0.927 was used. The results are given in percentage of glycogen in the fresh muscle.

RESULTS. The decrease in blood sugar following hepatectomy was in every case accompanied by a corresponding decrease in muscle glycogen which, although marked, never amounted to absolute disappearance.

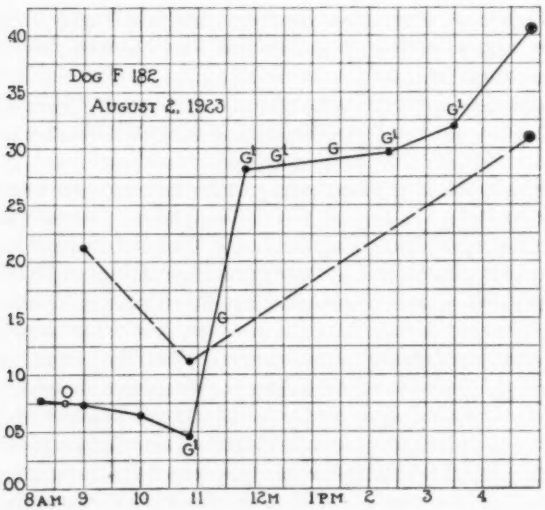

Fig. 1. Blood sugar (straight line) percentage and average glycogen content (dotted line) of the muscles in percentage following removal of the liver, *O*. Intravenous injection of 1 gram of glucose for each kilogram of body weight, G_1, and introduction of a similar amount of glucose into a jejunostomy tube *G*.

The symptoms of hypoglycemia appeared at a definite level of blood sugar (0.035 to 0.025 per cent), but in the different animals the absolute amount of glycogen in the muscle bore no definite relation to the symptoms. Symptoms appeared in some animals when the glycogen content of the muscles was 0.35 per cent, and in others not until the glycogen content had fallen to 0.10 per cent. However, there is a relation between the glycogen content of the muscle at the time of hepatectomy and the appearance of symptoms of hypoglycemia. Those animals with a high glycogen content did not manifest symptoms as early as those with a low glycogen content. An animal with a glycogen content of 0.75 per cent survived

hepatectomy ten hours before showing symptoms. An animal with 0.21 per cent of glycogen went but two hours without symptoms.

On injecting glucose to maintain a hyperglycemia after the muscle glycogen had been allowed to diminish, there was definite reformation of glycogen in the muscles. Considerable variation in the amount of glycogen reformed was observed in different muscles and even in the same muscles from opposite sides of the body. But in every case, there was a definite increase in muscle glycogen after the hyperglycemia had been maintained for a few hours.

In those experiments in which a hyperglycemia was maintained by repeated injections of glucose immediately after removal of the liver, the glycogen content of the muscles decreased during the first few hours, but

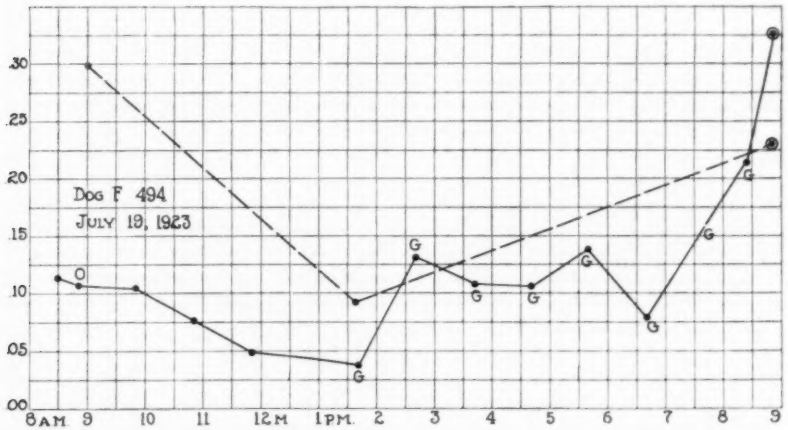

Fig. 2. Blood sugar in percentage and average glycogen content of the muscles in percentage, following removal of the liver, *O*. Intravenous injection of 1 gram of glucose for each kilogram of body weight, *G*.

subsequently increased. This finding is similar to that of other observers who have followed the formation of glycogen in the intact animal, in that several hours were necessary for the formation of glycogen from glucose.

That glycogen is utilized by conversion to glucose in the dehepatized animal was demonstrated in the experiments in which glycogen[1] was injected intravenously in animals comatose from the hypoglycemia following hepatectomy. There was an immediate recovery from symptoms and

[1] Glycogen was prepared from dog livers by Pflüger's method, and purified by repeated washings in alcohol and ether. It was then dried as a white amorphous powder, which contained no glucose or other reducing substances, and but a trace of nitrogen.

the animal could walk and seemed normal within five minutes after injection. The glucose of the blood was increased almost the same as if glucose had been injected. This demonstrated the presence of an active glycogenase present in a dog several hours after removal of the liver.

The utilization of glycogen by the hypoglycemic animal following hepatectomy differed from the reported inability of glycogen (in another species of animal, however) to prevent symptoms of hypoglycemia after the administration of insulin (Noble and Macleod, 1923). We deemed it advisable to determine the effect of glycogen on the symptoms of insulin hypoglycemia in dogs. On intravenous injection of glycogen (the equiva-

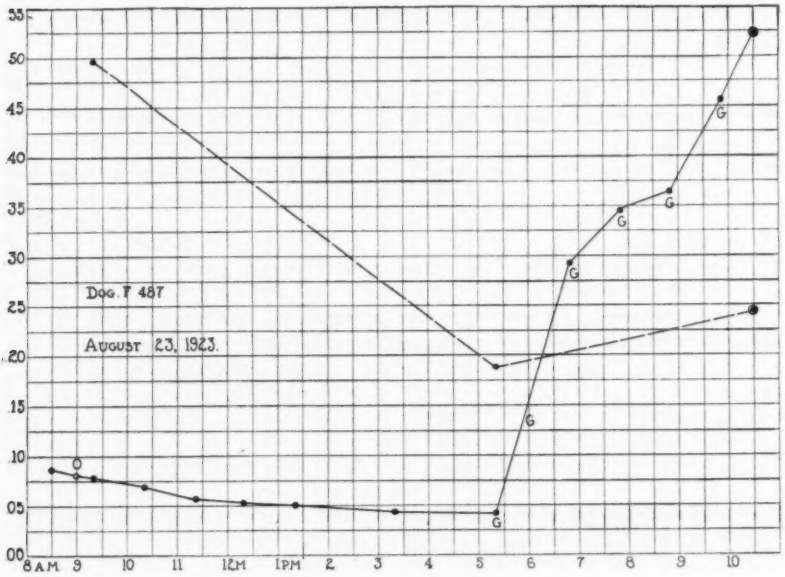

Fig. 3. See legend for figure 2

lent of 0.5 gram glucose for each kilogram of body weight) the animal, comatose from insulin, promptly returned to normal, and the blood sugar increased in a similar manner as though glucose had been given. Normal animals injected intravenously with glycogen give a blood sugar curve similar to the curve given by the injection of similar amounts of glucose.

Discussion. Although the total amount of glycogen in the muscles is as great as, or greater than, that in the liver, muscle glycogen plays a very minor part in the regulation of the blood sugar. None of the mechanisms which produce hyperglycemia in the normal animal (epinephrin

(Cannon, 1914), asphyxia (Pavy, 1899), excitement (Scott, 1914; Shaffer, 1914) and so forth) produce any noticeable effect following hepatectomy (Mann and Magath, 1925). The dehepatized animal does not liberate

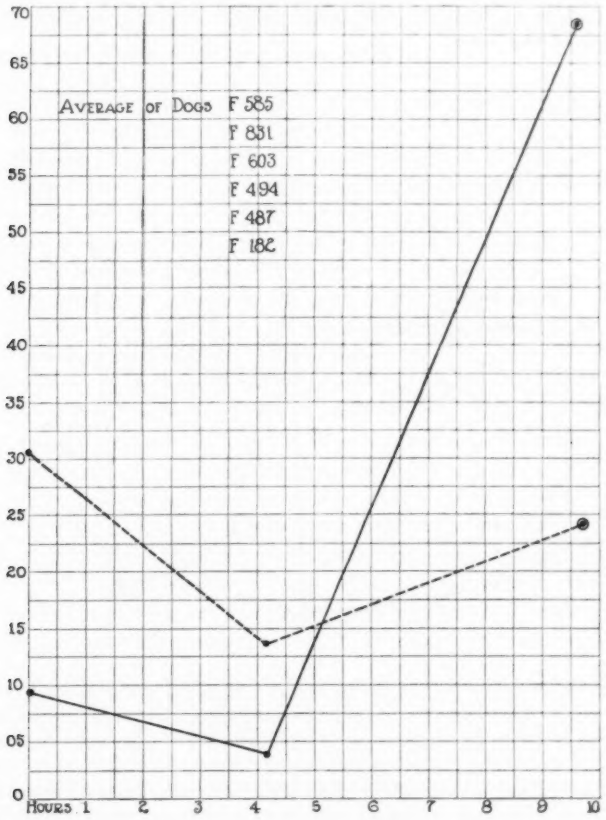

Fig. 4. Average decrease in blood sugar and decrease in muscle glycogen following complete removal of the liver at *O*. The increase in blood sugar and muscle glycogen was obtained after an average time of four hours by intravenous injection of 1 gram of glucose for each kilogram of body weight for each hour.

muscle glycogen fast enough to prevent the early development of hypoglycemia.

This inability of the muscle to liberate glycogen quickly enough to prevent death from hypoglycemia in the dehepatized animal is in marked contrast with the rapid rate of conversion of muscle glycogen to lactic

acid by muscular activity (Myerhof, 1924). Muscle glycogen certainly does not replace hepatic glycogen and the hepatic mechanisms by which the normal level of blood sugar is maintained. It would seem as if the glycogen stores of the muscles were mainly reserved for muscular activity and the supply of sugar to the blood almost entirely dependent on the functioning of the liver.

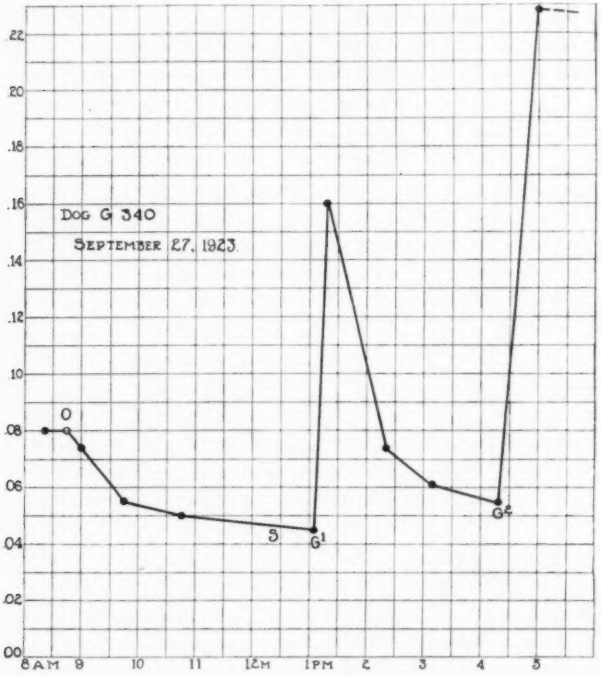

Fig. 5. Decrease in percentage of blood sugar following complete removal of the liver, *O*. At *S*, symptoms of hypoglycemia were present and were entirely dispelled three minutes after intravenous injection of a solution of glycogen equivalent to 0.5 gram for each kilogram of body weight at G_1. At G_2, symptoms of hypoglycemia were again dispelled by the intravenous injection of 1 gram of glucose for each kilogram of body weight.

Glycogenase is present in the blood several hours after hepatectomy and is sufficient in amount to hydrolize injected glycogen and restore the hypoglycemic animal to normal. The coincident presence of this glycogenase in the blood and of glycogen in the muscle, even at a time when its hydrolysis would allow the animal to survive, is evidence that the

glycogenase does not act freely within the cell. Either the glycogenase does not enter the cell freely or there is some inhibitory agent within the cell. Apparently there are other factors in the liberation of muscle glycogen as glucose into the blood stream since glycogen once circulating in the blood is immediately converted into glucose in those animals whose blood contains glycogenase (Compton, 1923).

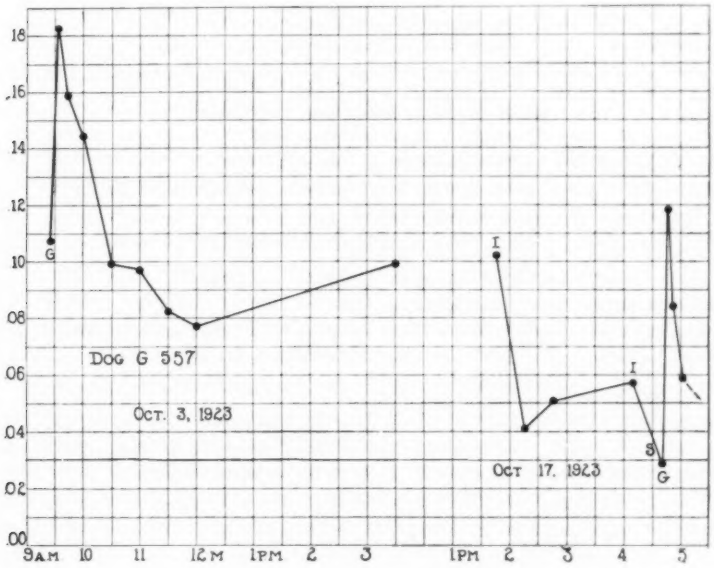

Fig. 6. Blood sugar following intravenous injection, *G*, of glycogen solution, equivalent to 0.5 gram of glucose for each kilogram of body weight in a normal dog. *I*, intravenous injection of one unit of insulin for each kilogram of body weight. Symptoms of hypoglycemia, *S*, were promptly dispelled by the intravenous injection of a solution of glycogen, *G*, equivalent to 0.5 gram of glucose for each kilogram of body weight.

SUMMARY

In dehepatized dogs the amount of glycogen in the muscles decreases as blood sugar decreases. Glycogen injected intravenously is converted into glucose and utilized by the hypoglycemic animal. The symptoms which appear with a definite hypoglycemic level (0.035 to 0.025 per cent) bear no relation to the absolute amount of glycogen in the muscles, and appear while there is still sufficient glycogen to bring the blood sugar to normal if it were released. The glycogen in the muscles is incapable of rapid conversion to glucose to maintain the level of sugar in the blood.

The liver must be regarded as the source of glucose in the blood, and the muscles play little or no part in maintaining the normal level of blood sugar. Following injections of glucose these hypoglycemic dehepatized animals immediately recovered, and if administration of glucose was continued for some time, muscle glycogen was reformed.

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AN INHIBITION IN OVULATION IN THE FOWL BY THE INTRAPERITONEAL ADMINISTRATION OF FRESH ANTERIOR HYPOPHYSEAL SUBSTANCE

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In 1915 Clark reported that the feeding of dried, powdered anterior lobe hypophyseal substance increased egg production in the domestic fowl. This stimulating effect of pituitary administration upon egg production in the fowl has not been confirmed by others, however. It has been shown that the intraperitoneal injection of small amounts of dried anterior lobe substance failed to activate the resting ovary, apparently lengthening the resting period (Pearl and Surface, 1915). The administration *per os* of this substance was without effect when egg production was declining, and sexual maturity also was not influenced (Pearl, 1916). Simpson (1923) failed to confirm the findings of Clark, egg production being unaffected by the feeding of dried anterior pituitary.

Since experimental evidence points quite clearly to the fact that the enteral administration of anterior lobe substance in the mammal is without effect, it is not surprising that the conclusions of Clark have not been confirmed. Nor is it surprising that the intraperitoneal administration of dried anterior lobe substance gave only uncertain results, for it has been shown in this laboratory that this treatment is ineffective in the mammal and in the tadpole. The work of these investigators did not show, however, that the anterior lobe hormones may not have a profound effect upon ovulation in the fowl. The work of Evans and Long (1921) in which it was shown that *fresh* anterior pituitary substance injected intraperitoneally profoundly stimulated growth and inhibited ovulation in the rat, makes it seem probable that effects upon ovulation would also be produced by this treatment in the fowl. This probability made it seem worth while to institute the experiments here described. Indeed, because of the fact that the egg appears externally in the hen, it seemed that this material might be of considerable value in a study of the ovarian response to a potent anterior hypophyseal extract.

For the experiment, White Leghorns were selected with care from the

[1] Aided by a grant from the Research Board of the University of California.

pens of a fancy poultry breeder. Each hen came to us with a trap nest record for the previous year, our experiment being performed in the second year of egg production. Their records showed good production—an average of 160 eggs for their pullet years.

At the laboratory they were kept out of doors in pens about 4 by 12 feet with a cement floor covered thickly with straw. The roof was canvas, which permitted rolling up during the day and adequate protection was afforded in inclement weather. The twenty hens used in the experiment were arranged in two pens of ten each with three trap nests to the pen, or approximately one nest for three hens. Food troughs were provided for dry mash (Sperry Surelay), charcoal, grit and oyster shell. In addition, they were fed a pan of wet Sperry mash every morning and Sperry Scratch feed morning and night. Lettuce or green grass was supplied daily in addition.

Four substances were used for injection.

1. Sterile Locke's solution. This control liquid was used to determine the animal's response to the handling incident to the experiment.

2. Muscle extract. This, used to determine the animal's reaction to the shock of protein absorption, was prepared by grinding with sand, fresh bovine cheek muscle, which had been sterilized for ten minutes in 40 per cent alcohol, after which it was diluted with three times its volume of salt solution and centrifuged. The supernatant fluid, which was fairly rich in protein, was then ready for injection.

3. "Standard" anterior hypophyseal substance, and

4. An "aqueous" anterior hypophyseal extract, substances which were prepared as follows:

The anterior lobes of beef pituitaries, received not longer than six hours after the death of the animal, were sterilized in 40 per cent alcohol for ten minutes, ground with sterile sand and diluted with one-third their volume of sterile saline solution. The supernatant fluid secured from centrifuging this "bowl" mixture comprised the "standard" mixture referred to above under "3". It was injected not later than twelve hours after the beef was killed. The "aqueous" extract (substance no. 4) was prepared by adding an equal volume of 8 per cent alcohol to the ground diluted glands ("bowl mixture") before centrifuging. After shaking for two hours, this mixture was allowed to stand overnight on ice; the shaking allowed a maximum denaturation and the standing a maximum agglutination. After centrifuging for one hour the clear, red-tinged liquid somewhat freed from protein, was evaporated down *in vacuo* at room temperature to the original amount of "bowl" mixture used, and was ready for injection. For example, to 15 grams of ground anterior lobes there would be added 5 cc. of saline solution and to this "bowl" mixture 20 cc. of 8 per cent alcohol were added. This would later be evaporated back to 20 cc., approximately

the original volume. This "aqueous" extract, the fourth substance used, seemed best suited for our purpose because of its lower protein content and because it seemed to require a smaller dosage to produce results than did the "standard" mixture.

It will be noted that the "standard" mixture was injected at most twelve hours after the death of the animal, while the "aqueous" extract was not injected for thirty-six hours. The animals were injected about ten o'clock each night. Three groups of experimental animals were run.

Group I. The first experimental group consisted of two animals, one an uninjected control, the other a "standard" pituitary injected animal (table 1). A pre-experimental record had shown an average egg production.

The injection of "standard" pituitary substance here completely inhibited egg laying for the period of the injection after the two eggs were laid

TABLE 1

Showing egg production of a "standard" pituitary injected hen (2 cc. per day) and its untreated control (group 1)

FOWL	PRE-INJECTION PERIOD			INJECTED PERIOD		
	Days	Eggs laid	Average/day	Days	Eggs laid	Average day
No. 525, untreated control	21	15	0.71	36	25	0.70
No. 105, "Standard" pituitary injected	21	14	0.66	36	2	0.055

which are normally found in the oviduct of a laying fowl.[2] That there is an immediate effect upon the developing ova, although they may be approaching maturity, is shown here by the fact that no more eggs were produced during the injection period. Regressive changes (atresia) lead to the disappearance of these yolk masses even though they be large, as will be shown later, thus pointing to an immediate effect upon the ovary. In addition to the stoppage of laying the injected animal was thrown into a severe moult.

These two animals exemplify the behavior of all our experimental animals; their reactions can be briefly stated as follows: 1, Control animals continued to lay without interruption throughout the experiment, while 2, pituitary injected animals ceased production after laying the two or three eggs present in the oviduct when treatment was commenced.

Group II. This consisted of seven animals, the experimental injections

[2] Later work showed that occasionally three eggs were deposited.

beginning on March 8. Six of these were put on a sequence consisting of an un-injected, a saline injected, and a muscle extract injected period, these constituting the control period, which was followed by a period of pituitary injection. The seventh animal received no injection throughout the experiment. The various treatments were carried out in unbroken sequence, control substances being injected approximately for two weeks, while pituitary injections extended for a period of 20 to 84 days. The results obtained from this experiment are shown in table 2, which summarizes the daily record by periods.

TABLE 2

Showing by periods, the egg production of fowls under a regime of saline, muscle and anterior pituitary injections after a preliminary period during which no injections were made

FOWL	SUBSTANCE INJECTED											
	None			Saline			Muscle			"Standard pituitary"		
	Days	Eggs	Average	Days	Eggs	Average	Days	Eggs	Average	Days	Eggs	Average
736	20	12	0.6	34	21	0.6	14	11	0.8	20	16	0.8*
381	20	12	0.6	34	20	0.6	14	12	0.8	20	2	0.1
										Pituitary "aqueous" extract		
394	7	3	0.4	7	3	0.4	6	3	0.5	34	4	0.12*
221	7	4	0.6	7	4	0.6	6	4	0.6	67	5	0.07*
54	18	11	0.4	18	9	0.5	18	12	0.6	36	3	0.07
274	7	4	0.6	7	4	0.6	6	4	0.6	80	19	0.2*
739	No injections									100	37	0.33
										(no injection)		

* These animals resumed laying during the experiment but were stopped by increased dosage.

An exception might appear to be shown by four of these animals to our earlier statement that anterior pituitary injections inhibit laying after the two eggs normally present in the oviduct are laid. This apparent exception only points, however, to the importance of the dosage since in all cases complete inhibition in laying was obtained by increasing the dosage. Of the two animals of this group receiving "standard" anterior lobe, laying was inhibited in one by a dosage of 2 cc. per day, while one required 4 cc. per day to stop laying. Of the animals receiving the "aqueous" pituitary extract, one was stopped by the injection of $\frac{1}{2}$ cc. per day, while in three cases this had to be increased to 1 cc. per day. One animal, no. 736, indeed proved most refractory, laying through most of the period of injection. Egg production was finally stopped however. In no case was a toxic effect evident.

In group III five other animals were given identical treatment as regards dosage and time. After a preliminary control period during which no injections were given, saline solution was injected for 14 days, followed by muscle extract for the same period of time, at the termination of which pituitary anterior lobe substance was injected for twenty days. The results by periods which were obtained from this group are shown in table 3.

It will be noted that the animals, all of which had been laying normally during the control periods, were completely inhibited within five days after the first hypophyseal injection, during which time the two or three eggs

TABLE 3

Showing by periods the egg production during a preliminary observation period, a period of saline, of muscle, and of pituitary injection and the time lapsing before laying was resumed (group III)

FOWL	SUBSTANCE INJECTED												
	None			Saline			Muscle			"Standard" pituitary injection			No injection
	Days	Eggs	Average	Days	Eggs	Average	Days	Eggs	Average	Days	Eggs*	Average	Number of days before laying was resumed†
661	17	10	0.6	15	11	0.7	14	10	0.7	20	1	0.05	15
325	17	12	0.7	15	8	0.5	14	9	0.6	20	3	0.15	72
449	17	8	0.5	15	11	0.7	14	10	0.7	20	2	0.1	13
254	17	12	0.7	15	10	0.6	14	7	0.5	20	3	0.15	46
160	17	10	0.6	15	5	0.3	14	8	0.55	20	1	0.05	26
Averages...	17	10	0.6	15	9	0.56	14	9	0.6	20	2	0.1	

* In all these cases the eggs were laid during the first five days after pituitary injections were started.

† All the animals laid at a normal rate after production was reëstablished.

normally present in the oviduct were laid. That the inhibition in laying affected by anterior lobe injection was not due to the toxic action of this substance is shown by the fact that weighings made at the conclusion of the experiment in no case show a loss in weight, a slight gain being made in a few cases. Furthermore, no effects, such as were noted by Pearl and Surface, i.e., rapid and difficult breathing, and illness resulted from the injections.

To further check the results which seem to indicate a specific action exerted by the anterior lobe of the hypophysis upon ovarian function, histological studies were made of the ovaries of two animals not receiving and of two animals receiving pituitary injections of this substance. Both control animals showed an unbroken laying record, while the pituitary injected animal had stopped laying some time before being sacrificed.

In one of the controls (no. 525) two fully formed eggs were found in the oviduct. There were three apparently fully formed yolks attached to the ovary with numerous ova ranging in size from 12 mm. down to microscopic dimensions. Besides these there were many whitish or yellowish bodies which have been described as corpora lutea. The entire ovary of this animal was fixed in formol and serially sectioned. In the other control (no. 683) there was one egg in the oviduct, two yolks of about full size in the ovary, two half developed and many of smaller dimensions. Three apparently recently ruptured follicles lying on the surface of the ovary presented a fan-like, flat appearance.

A microscopic study of the sections of the normal ovary revealed yolks of varying dimensions, most of which were typical showing no signs of atresia, and corpora lutea or their remains.[3]

The pituitary injected animals on autopsy presented a clean abdominal cavity, free from any signs of infection or adhesion, and with a characteristic fat pad. The ovaries were strikingly smaller than in the normals, and presented a beaded or nodular appearance, due to the great numbers of small follicles and of yellowish bodies which appeared to differ in no respect from the degenerated corpora lutea of the normal hen. It appeared significant as indicating atresia, as has subsequently been confirmed by a study of the sections, that the follicles of larger dimensions (7 to 10 mm.) were soft and filled with a material semi-fluid in consistency, in contrast to the firm character of these structures in the untreated controls. No follicles exceeding 10 mm. in diameter were present.

Microscopic examination of the pituitary treated animals revealed degenerative changes in most of the small as well as in the larger ovarian follicles. There was clearly a liquefaction and a cellular invasion of the yolk in all ova which had attained a diameter of 7 mm., a change which is characteristic of atresia (Pearl and Boring, 1918). It appears that this degeneration prevented the formation of yolks of large size, the absence of which was a noteworthy feature of the pituitary injected fowls.

Microscopically Evans and Long (1922) found that the ovaries of rats injected with anterior lobe substance were filled with corpora lutea, and were larger than normal. In hens we found numerous yellowish bodies and small follicles but the size of the entire ovary was greatly dimin-

[3] Corpus luteum formation in the fowl has been questioned by Nonídez (1922, who with others, Novak and Duschak (1923), Fell (1924), believes that the cells described by Pearl and Boring (1918) as "luteal" are not true luteal cells. Novak and Duschak, whose findings are in harmony with those of Hetts (1922) show rather convincingly that all the characteristic elements of a corpus luteum are present, and state that a rudimentary corpus is formed. We therefore feel justified in using the term "corpus luteum." For the anatomical literature on the subject the reader is referred to the above mentioned paper by Novak and Duschak.

ished, as compared to the normal controls. The fact that the ovaries of the injected birds are decreased in size in contrast to the ovaries of the injected mammal, can be explained by the fact that the bodies which form from the follicular degeneration in the avian ovary are relatively much smaller than the corpora of the mammal, and comprise but an insignificant part of the bulk of the ovary, the immense size of the functioning ovary of the fowl being due to the gigantic ova.

Discussion. Results of our work show decidedly that fresh anterior hypophyseal substance when injected intraperitoneally, inhibits ovulation in the fowl. This inhibition is not due to the handling of the fowls nor to any shock of protein absorption as shown by control experiments. The explanation of the difference in the findings of Clark, of Pearl and Surface, and of Simpson as contrasted to our findings appears to lie in the method of administration and the freshness of the hypophyseal substance used, factors whose importance has been demonstrated beyond question by Evans and Long. These authors showed that fresh anterior hypophyseal substance injected intraperitoneally caused an unmistakable increase in growth and inhibited oestrus and ovulation in the rat, effects which, in common with many other investigators, they failed to secure by the oral administration of either the fresh or dried substances. The Smiths (1922) working with the tadpole, have also shown that the thyroids and interrenal glands which are atrophic as a result of hypophysectomy, the animals not metamorphosing, can be brought to a normal structural condition and the animal metamorphosed by the injection of anterior lobe substance, an effect which cannot be secured by oral administration.

It may seem paradoxical that operations upon the hypophysis also inhibit ovulation and oestrus, as shown by the work of Paulesco (1906), Aschner (1909), Cushing (1909) and later Smith, Walker and Graeser (1924) and others. However similar these physiological effects upon ovulation may seem to be, the anatomical picture presented in the mammal by the two types of ovary is not similar. In the pituitary injected animals there is formed an abundance of lutein tissue, a tissue which is largely or entirely lacking in the hypophysectomized animal.

The abundant lutein tissue invariably found in the ovaries of the pituitary injected rats led Evans and Long to conclude that "a powerful, specific stimulus to lutein cell transformation has thus been effected by this hormone." That the abundance of the lutein tissue might be the factor responsible for the inhibitions of ovulation would receive support from the work of Loeb (1914) who found that the reverse condition, namely, that destruction of the corpora hastened the appearance of the next ovulation; from the work of Pearl and Surface (1915) who found that the injection of dessicated corpus luteum inhibited ovulation in the hen, a finding which has not, however, been supported by Corner and Hurni (1918) in the rat, and

by the fact long known to the veterinarians that the manual expression of a corpus would bring on oestrus in an animal in which this phenomenon was long overdue. From our finding in the fowl, however, we are inclined to believe that, here, ovulation is not inhibited by a deterrent influence exerted by any lutein tissue hormone, but rather by the degenerative or atretic processes which are induced in the developing follicle by the injected anterior lobe substance. We have pointed out that the developing ova even in their earlier stages changed from their normal firm consistence to a soft semi-fluid condition. Thus any formation of lutein tissue must succeed and not precede the breakdown of the ova. It thus appears that in the fowl an excess of the hormone exerts an injurious influence directly on the maturing ova, rather than inhibiting ovulation through the medium of corpus luteum formation.

The writer wishes to express his appreciation for the aid given by Dr. P. E. Smith, at whose suggestion this work was undertaken and under whose direction it progressed.

SUMMARY

1. The intraperitoneal administration of fresh anterior hypophyseal substance inhibits ovulation in the domestic fowl.

2. This inhibition in ovulation is not due to any general systemic toxic effect since the fowls remained in excellent health and in most cases gained in weight. It appears rather to be due to an injurious effect exerted by the excess of the hypophyseal hormone upon the developing ova.

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EFFECTS OF VARIATION IN FREQUENCY OF STIMULATION OF SKELETAL, CARDIAC AND SMOOTH MUSCLE WITH SPECIAL REFERENCE TO INHIBITION

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Inhibition of the smooth muscle of parts of the alimentary canal of the cat, by stimulation of the vagus nerve, resembles so closely Wedensky inhibition (1) that added interest is given to the question whether an inhibitory reaction can be evoked by increasing the frequency of direct stimulation of muscle. A considerable number of previous observations indicate that such is the case, but little effort has been made to demonstrate that the more or less complete relaxation of muscle, in response to a relatively high frequency of stimulation, is of the nature of true inhibition. It was with this purpose that the present investigation was undertaken. Preparations of skeletal, cardiac, and smooth muscle were subjected to different frequencies, and intensities incidentally, of direct faradization, and in the case of the first two varieties, the inhibitory effects of the higher frequencies, particularly in regard to rhythmicity, were further studied by the use of atropine. Most of the experiments were carried out at the Harvard Medical School, but others were performed at Cambridge University for the purpose of determining the extent of propagation of the effects of the higher frequencies.

PREVIOUS OBSERVATIONS. Bernstein (2) obtained the initial contraction effect by stimulation of the curarized sartorius of the frog with the same frequencies which had given it on indirect excitation of the gastrocnemius. Richet (3) observed a similar phenomenon on faradization of the muscles of the crayfish claw. Kronecker and Gotch (4) found, furthermore, that the rate of fall of tetanus curves of muscles of the rabbit, and of the curarized or non-curarized frog, was proportional to the frequency of direct stimulation. Likewise, Wedensky states in his Russian monograph (5, §79, p. 188, and résumé, no. 26) that the curarized gastrocnemius reacts to variation in frequency, but not in intensity, in much the same way as the indirectly stimulated muscle, and at times in a more pronounced

[1] A preliminary report of the work done at Harvard was published in Science, 1924, lx, 225.

manner. With the non-curarized sartorius, he obtained relaxation by increasing both the frequency and the intensity, provided that the induction shocks were not too strong (6). More recently, Tullio (7) has observed the initial contraction effect on direct stimulation of both non-curarized and curarized muscles with moderate intensities, whereas weaker or stronger stimulation failed to produce it.

Somewhat similar phenomena have been observed also on direct stimulation of cardiac muscle. Engelmann (8) found that each induction shock applied to the isolated ventricle of the frog's heart at intervals of two or more seconds produced a contraction. If the interval between the shocks was less than one second, however, only the first evoked a systole, those following causing at most a weak local action. Basch (9) reported, soon afterwards, that stimulation of the frog's ventricle with induction shocks in rapid sequence evoked only a single contraction, or a brief series of contractions, whereas lowering the frequency caused it to resume its activity. Ranvier (10) states, likewise, that faradization of this preparation with tetanizing frequency, but moderate intensity, leads to relaxation of the muscle after an initial contraction. Very strong currents, on the contrary, produced a tonic shortening and minimal shocks evoked rhythmicity. Lahousse (11) found later that stimulation of the frog's heart after exclusion of the sinus by ligature, or of the isolated apex of the ventricle, with a sufficiently high frequency of induction shocks, resulted only in an initial systole. Similar results have been obtained by Erlanger and Garrey (12) from strips of the turtle's ventricle.

In regard to smooth muscle, Engelmann found that the conductibility for a wave of contraction in the ureter of the rabbit was lowered after the application of an effective stimulus, whether mechanical (13) or a brief voltaic current (14). Repetition of the stimulus sufficiently soon after the passage of the first wave of contraction had only a local effect, and in case a series of currents of sufficient frequency was applied, the local contraction was limited to the cathode. Bottazzi (15) often obtained an initial contraction effect on direct faradization of the esophagus of Aplysia. Schultz (16) observed, furthermore, that the contraction of a ring from the frog's stomach, in response to a series of induction shocks, rose more steeply and fell more quickly during stimulation, the briefer the interval between the stimuli. Mislawsky (17) obtained similar results. The after-effects of stimulation, shown in some of his tracings, resemble reactions of parts of the alimentary canal of the cat following excitation of the vagus (1).

EXPERIMENTS PERFORMED AT HARVARD. *Experimental methods.* The electrical apparatus employed in stimulation has been described in a preceding paper (1). Coil B, with its automatic interrupter, was used only in faradization of the siphon muscle of the clam. In all other experiments, the rotary interrupter and coil A served, the duration of closure of the

primary circuit being regularly 50 per cent for frequencies of interruption below 120 per second, and 60 per cent for higher frequencies. The primary current was adjusted to 0.1 ampere, except in stimulation of relatively inexcitable preparations of the apex of the ventricle of the tortoise, when the current density was increased. Neither make nor break shocks were short-circuited. As a rule platinum or silver wires, thrust through the opposite ends of the excised preparation, were employed as stimulating electrodes. They were connected with the poles of the secondary coil by means of fine copper wires, which did not interfere appreciably with the form changes of the muscle.

The gastrocnemius of the frog, taken usually from Rana temporaria, the omohyoid and the apex of the ventricle of common fresh water tortoises, Pseudemys concinna being selected most often, portions of the alimentary canal of the cat, and the siphon muscle of the clam were subjected to faradization. The apex of the ventricle of the tortoise was suggested as the representative of cardiac muscle by one of us (J. R. P.) particularly because of its probable freedom from vagus action. As a regular procedure, the animals were anesthetized, or, as in the case of the frog and tortoise, the brain was destroyed before operation was begun.

Curarization of frogs was effected usually by injecting doses of 0.5 to 0.8 cc. of 1 per cent curare solution, for animals of 40 to 50 grams weight, into the dorsal lymph sac. Atropinization was brought about similarly by injecting doses varying from 1.5 to 3.0 cc. of 0.2 per cent of the sulphate in Ringer solution. The animals were allowed to rest an hour or more thereafter. In case it was desired to protect a gastrocnemius from the action of one or both drugs, a ligature was applied tightly about the corresponding thigh before injection. To denervate the muscle, the sciatic was cut in the thigh, under ether anesthesia, and with aseptic precautions, the cut ends of the nerve were separated by a few millimeters, and the wound was closed with silk sutures. The animal was placed then in cool, running water, and time was allowed for degeneration of the nerve.

For curarization of the tortoise, an average dose of 2.5 cc. of 1 per cent curare solution, for animals of about 650 grams weight, was injected, usually into an external vein of the neck. The apex of the ventricle was removed by a transverse incision between the middle and lowest thirds of the chamber. Threads were attached to the lateral extremities of the base of the pyramidal portion of cardiac muscle thus obtained, one being used to anchor the structure and the other to attach it to the recording lever. Atropinization was effected at first by dropping on the preparation 0.2 per cent atropine sulphate in Ringer solution. Pilocarpine hydrochloride, 2.0 per cent in Ringer solution, was added in the same way. Later, in order to insure more complete action of these drugs, the tissue was immersed in their oxygenated solutions, of the concentration previously employed, for a period of 15 minutes or more.

The portions of the alimentary canal of the cat consisted of the lowermost 2 cm. of the esophagus, and Magnus preparations (18) of the circular coat of the small intestine. These preparations were kept, as a rule, in a chamber of oxygenated Ringer solution, surrounded by a water bath near 37°C. For stimulation, the Ringer solution was siphoned off temporarily. The lower end of the esophagus was arranged for recording its longitudinal contractions. In the case of the siphon muscle of the clam, transverse sections of several millimeters thickness were prepared and arranged for recording the lateral contractions, one lateral extremity being fixed and the other being attached to the muscle lever.

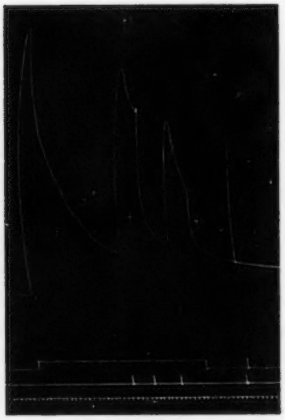

Fig. 1

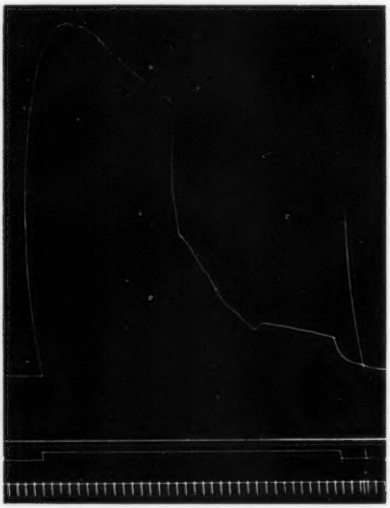

Fig. 2

Fig. 1. Curarized omohyoid of tortoise. Frequencies: 120; 20; 120; 20 per second; changes indicated by upstrokes in middle signal line. 370 Z units. Time in 5-second intervals in this and following figures, unless otherwise indicated.

Fig. 2. Curarized omohyoid of tortoise. Frequencies: 20; 40; 20 per second; changes indicated by upstrokes in uppermost signal line. 370 Z units.

In order to test the completeness of the curarization of the gastrocnemius and the omohyoid, and the denervation of the former, their supplying nerves were either faradized or stimulated mechanically or subjected to both procedures. In all experiments on these muscles and the apex of the ventricle, the preparations were arranged in a moist chamber at room temperature. The Harvard muscle lever was employed for recording the contractions of all preparations with the exception of those from the alimentary canal of the cat and some of the apex of the ventricle. For these,

the lightly loaded Harvard heart lever was used. The additional load for the gastrocnemius was 10 grams, and for the omohyoid, from 3 to 5 grams. The levers were so arranged that contraction was registered by an upward movement of the writing points. The accompanying figures read from left to right. Time is recorded in 5-second intervals. In stating the intensity in Z units (19), and the frequency per second of stimulation, only the break shocks are considered.

Results from the omohyoid and the gastrocnemius. Direct stimulation of the omohyoid muscle of the tortoise with relatively low tetanizing frequencies evokes a well-maintained contraction. With stimuli of sufficient intensity (300 Z units), frequencies of 20 to 40 per second keep the fresh muscle in a state of contraction, diminishing only gradually, for periods of minutes (fig. 2). Relaxation takes place more rapidly during stimulation with the higher frequencies, however, the rate of fall being more or less proportional to the frequency. Thus, with shocks at sufficiently brief intervals, the contraction takes the form of an initial tetanus, the relaxation being nearly complete after the first minute of stimulation (fig. 1). A certain amount of contraction usually persists, however, till cessation of faradization. The reaction occurs with somewhat lower frequencies for the curarized than for the non-curarized preparation, and it is promoted by the onset of fatigue. In a relatively fresh non-curarized muscle, for example, 480 interruptions per second (265 Z units) were required to produce the initial tetanus. Following this period of stimulation, however, the position of the secondary coil remaining unchanged, the corresponding effect was evoked by half the frequency. In the moderately fresh curarized muscle, a frequency of 120 is usually sufficient to bring about this reaction (fig. 1), whereas 40 per second may produce it in the fatigued preparation (370 Z units in a particular observation). The initial contractions, after many periods of stimulation, moreover, are decidedly submaximal in height, whereas those from fresh preparations are more nearly maximal. Their duration is decreased also by advanced fatigue. The reaction then may simulate a weak initial twitch followed by a more prolonged low grade of shortening. No trace of a final contraction was ever observed on interruption of the higher frequencies.

In correspondence with these results, a considerable increase in the interval between stimuli, when the muscle is more or less completely relaxed, leads immediately to pronounced and quite well maintained contraction. This effect is shown in figure 1, though the contraction often falls less rapidly than this tracing shows. Conversely a muscle in a state of marked tetanus can be made to relax by increasing the frequency (figs. 1 and 2). In curarized preparations the relaxation is preceded often by a brief increase in the shortening, tending to be more pronounced the lower the preceding tetanus (fig. 1). When this does not occur, the latent period for the relaxa-

tion is probably not more than one second. It is possible thus to throw the muscle into rhythmic contraction by alternately increasing and decreasing the frequency of stimulation.

The intercalation of a relatively high frequency between two lower frequencies of the same value, in a single period of stimulation, usually diminishes the magnitude of the contraction occurring on lowering the frequency. An extreme example of this effect is given in figure 2, where the response on changing from 40 to 20 per second is quite small. Some evidence was obtained that this diminishing effect might occur when the stimulation was begun by a higher frequency, though the contraction evoked by it was only a slight initial tetanus. In one non-curarized preparation, however, an increase to 480 per second (265 Z units) led to apparently complete relaxation and resulted in partial recovery from the fatigue caused by the preceding contraction.

The omohyoid of the tortoise displays these changes in reaction in a clear manner, for it has little tendency to enter into a state of contracture. The results as described above, moreover, were similar for all of the intensities employed, viz., from 205 to 530 Z units.

In general, the gastrocnemius of the frog reacts to variation in frequency of stimulation in much the same way as the omohyoid of the tortoise, though the relaxation produced by the higher frequencies appears to be diminished considerably by the occurrence of contracture. The phenomenon of rhythmicity, furthermore, is frequently exhibited by the gastrocnemius, though it was never observed on stimulating the omohyoid. In relatively fresh preparations, the lower tetanizing frequencies, as 40 per second, evoke a well-maintained tetanus. The fall in the contraction is quite gradual, extending over a period of minutes, if the stimulation is continued. Higher frequencies, however, produce contractions which fall more rapidly, the rate of relaxation during stimulation being more or less directly proportional to the frequency. Thus, for a given position of the secondary coil, a frequency of 80 per second produces a better maintained tetanus than one of 120, and the latter, a more lasting contraction than one of 270. The chief part of the contraction becomes limited more and more to the beginning of stimulation. For the last named frequency, its total duration may be little more than 10 seconds. After the occurrence of this initial tetanus, however, slight contraction persists till the end of stimulation. When a muscle is in such a partially relaxed state, a considerable fall in frequency, as from 120 to 40 per second, produces a pronounced and well-maintained increase in the height of contraction. This may be preceded, however, by a temporary fall in the curve. Conversely a corresponding increase in frequency leads to a partial relaxation of the tetanically contracted muscle. This extension is preceded usually, however, by a brief increase in the shortening, which seems to be more marked the

lower the degree of the preceding contraction. No indication of after-contraction was ever observed on interruption of stimulation with the higher frequencies. These remarks hold in general for non-curarized, curarized, and denervated gastrocnemii, and the results do not appear to be changed qualitatively with intensities varying from 145 to 495 Z units.

In five preparations (15 per cent of the total), the reactions changed somewhat after the onset of fatigue. All of them, when fresh, responded in the usual way. Two were taken from a frog apparently in good condition. One of these, which was non-curarized, in the third period of stimulation, responded at first to 20 per second (205 Z units) with maintained contraction, and an increase to 100 per second led to pronounced relaxation. Lowering the frequency thereafter, however, first to 20 and then to 10 per second, led in each case to slight relaxation. The other preparation, which was curarized, responded in a quite similar manner, in the second period of stimulation, to corresponding changes in frequency (495 Z units). Two other preparations were taken from a frog, the right sciatic nerve of which had been cut on January 17, 1924. On May 10, 114 days later, the animal was taken for experiment. It was poor in flesh, and it showed signs of weakness on exertion. The denervated muscle responded as usual during the first period of stimulation, but reversal effects were observed in the second. The most pronounced example of reversal, however, was obtained from the left gastrocnemius. In the first period of stimulation, lasting for 5 minutes, the muscle responded in the usual way to variation in frequency. In a succeeding prolonged period, a frequency of 320 per second (200 Z units) gave a fairly well maintained, but somewhat undulating tetanus. A decrease to 80 per second evoked a pronounced increase in the shortening, but this lasted only 10 seconds, the muscle thereafter relaxing to a level below that preceding the lowering in frequency. Alternations of 320 and 80 per second then resulted in rather uneven but well maintained contraction for the former, and incomplete relaxation for the latter. A less pronounced reversal was observed for lower frequencies in a fatigued atropinized preparation.

Very often in the course of prolonged stimulation of the gastrocnemius, the smooth tetanus at the beginning of the period changed gradually into a series of rhythmic contractions. This rhythmicity was exhibited in most striking manner by preparations, whether non-curarized, curarized, or denervated, from the common grass frog, Rana temporaria. It was essential, however, for the animals to be in good condition. The phenomenon was produced by all of the frequencies and intensities commonly used in the course of the experiments (from 15 to 120 per second and 145 to 530 Z units), though the contractions appeared to be stronger and to continue to recur longer for the lower frequencies. They first become manifest as a series of rapidly recurring undulations or irregularities in

the falling tetanus curve. Their frequency then may exceed 1 per second, though it was not definitely determined. As the contraction continues to decrease, these irregularities become more widely separated, more regular in their occurrence, and greater in their strength and duration until finally the curve rises and falls in a quite regular and well pronounced manner (figs. 3 and 4). Direct inspection of the muscle shows that the smaller irregularities are due to fibrillar twitchings. These gradually come to include more and more of the muscle, and eventually the whole preparation may appear to be involved in the rhythmic contraction. Such a series of contractions may persist for several minutes. If the muscle is somewhat fatigued, a pronounced rhythm may be produced at the beginning of stimu-

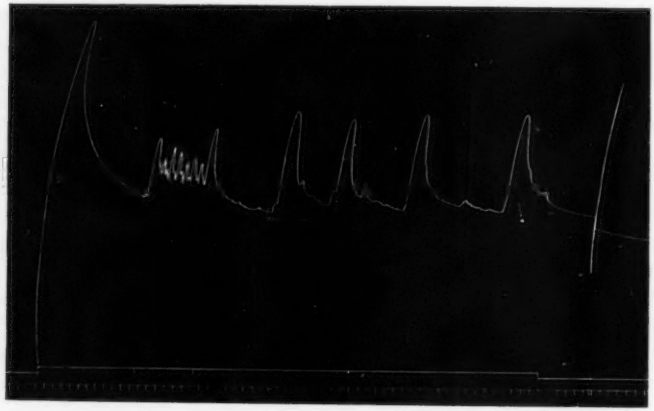

Fig. 3. Denervated gastrocnemius of Rana temporaria: sciatic cut 51 days previously. Frequencies: 120; 40; 120; 40 per second; changes indicated by downstrokes in stimulation signal. 370 Z units.

lation. The contractions themselves do not appear to differ greatly for different preparations, except that the period of shortening is more brief and abrupt in non-curarized than in curarized muscles and the one denervated preparation which exhibited them (fig. 3).

The occurrence of rhythmic contractions does not affect markedly the changes in the general level of shortening in response to variation in frequency. When rhythmicity has become established in response to 120 interruptions per second, a decrease to 40 results, as a rule, not only in a rise of the general level of shortening, but also in a lowering of the frequency and a marked increase of the magnitude and duration of the periodic contractions (fig. 3). They tend also to become more regular in their occurrence. At times this effect is preceded by a latent period of 5 to 18 seconds, duration (fig. 3), in which the muscle relaxes somewhat, but it may take

place immediately on change in frequency. On one occasion, it was given by a somewhat fatigued curarized muscle on a decrease from the relatively low frequency of 40 per second to one of 20 (145 Z units).

When rhythmicity has been established by a lower frequency, on the other hand, an increase to a higher frequency results, as a rule, in a well pronounced but brief augmentation in contraction, followed by relaxation to a level considerably below that preceding the frequency change and complete or nearly complete cessation of rhythmic contractions (fig. 3). Occasionally two or three contractions may take place while the muscle is relaxing in response to the higher frequency. This change in the reaction of the muscle occurs regularly for an augmentation of 40 to 120 per second, and for frequencies considerably lower if the preparation is fatigued. It was observed thus in a curarized preparation for an increase

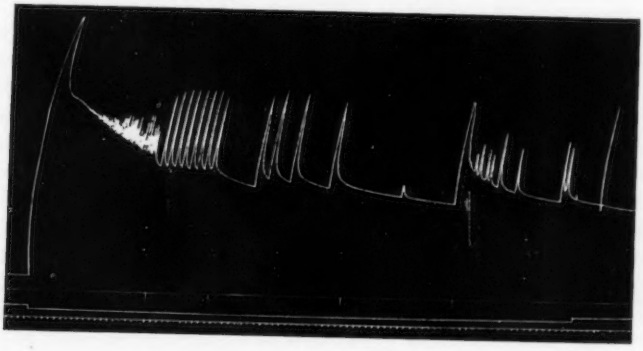

Fig. 4. Atropinized and curarized gastrocnemius of Rana temporaria. Frequencies: 320; 400; 480; 560; 400 per second; changes indicated by downstrokes in uppermost signal line. 205 Z units.

from 20 to 60 (145 Z units), and in a non-curarized muscle for an augmentation from 10 to 40 per second (205 Z units). In one observation on a curarized preparation, an elevation from 40 to 120 (145 Z units) resulted in apparently complete cessation of mechanical activity of the muscle, excepting the persistence of contracture, and in partial recovery from the fatigue produced by the preceding contractions. The latter was shown by the increased ability of the muscle to contract when the frequency was lowered to its preceding value.

Most of the changes in reaction enumerated above for variation in frequency of stimulation of the gastrocnemius are illustrated in figure 3. The sciatic nerve to the muscle, from which the tracing was taken, had been cut 51 days previously. Neither mechanical nor strong, tetanizing faradic stimulation of the degenerated nerve produced a mechanical

response. It is seen that 120 interruptions per second evoked strong contraction. The relaxation during stimulation was relatively rapid at first, and in the later and more prolonged part of its course, slight irregularities began to appear. Lowering the frequency to 40 per second not only resulted in a prompt rise in the general level of the shortening, but also in the appearance of rhythmic contractions. These were relatively close together and small in magnitude. An increase to 120 thereafter led to a transient increase in the contraction, followed by the usual relaxation to a level much below that preceding the change, together with nearly complete cessation of rhythmic activity. It is seen that the succeeding change to 40 per second was not followed by an immediate response of the muscle. The progressive relaxation continued for a period of 18 seconds before the first member of the series of strong rhythmic contractions appeared. With their occurrence, moreover, the general level of contraction rose somewhat. Evidence is given in this tracing also of the dependence of the size of the rhythmic contractions on the interval between them. The contractions of the second series in response to 40 per second are much larger than those of the first, and they are much more widely separated. The second of the larger contractions, furthermore, does not reach so great a height as its successor, and the period between these is greater than that which separates the former from the first of the series.

It was found that atropine increased the tendency of the gastrocnemius to respond rhythmically to faradization and opposed decidedly the inhibitory action of the higher frequencies. This was true for both curarized and non-curarized preparations. Thus, in stimulating the non-atropinized, non-curarized muscle from the right leg of a frog, an increase from 40 to 120 per second (secondary at 11 cm., 205 Z units), after producing a brief introductory increase of the shortening, led to pronounced relaxation and almost complete cessation of the rhythmic contractions previously in progress. The atropinized non-curarized preparation from the left leg, on the contrary, responded rhythmically to the highest frequencies employed, viz., up to 560 per second (secondary at 11 cm.) Increases to 400 per second, and above, caused the muscle to relax partially, and resulted for a brief period in almost complete disappearance of contractions. With continued stimulation, however, they reappeared with relatively great magnitude. In the experiment from which figure 4 was taken, stimulation of the atropinized, non-curarized gastrocnemius resulted in rhythmic contractions with frequencies up to 640 per second, the highest employed (secondary at 11 cm.). Increases above 400 per second were attended by the same series of events as described for the preceding similarly prepared muscle. The rhythmicity of the curarized and atropinized muscle from the opposite leg, however, almost completely disappeared with a frequency of 560 (secondary at 11 cm.) as shown in figure 4. In one other experiment,

curare apparently reduced somewhat the ability of the atropinized preparation to respond rhythmically to high frequencies. The drug showed a tendency also to make the contractions of the atropinized muscle more regular in size and in frequency of occurrence. The slowing effects of the higher frequencies on the contractions of non-curarized, atropinized muscles was obscured considerably by the occurrence of smaller contractions between the larger ones. These effects of decreasing the interval between stimuli are well shown for the curarized, atropinized preparation in figure 4. The failure of an introductory contraction to precede the partial relaxation in response to an increase in frequency, as illustrated in figure 4, held generally for atropinized gastrocnemii.

Results from the apex of the ventricle of the tortoise. When the apex of the ventricle of the tortoise is freshly excised and arranged for stimulation in a moist chamber, it remains quite inactive and usually atonic. Under these conditions it responds to a single induction shock with a single contraction. In two preparations (20 per cent of the total), the relaxation following the contraction thus produced fell slightly below the level preceding it. The original level was soon regained, however, and a slight degree of tonus was thus revealed. One of these preparations was taken from an animal which had received 15 cc. of 0.2 per cent atropine sulphate in Ringer solution intravenously. A series of shocks of the lowest frequencies employed in the present experiments, viz., 1.25 per second and upwards, depending on the condition of the preparation, produces a regular rhythm of contractions much slower than that of the stimulation. At the beginning of a period of faradization, they are likely to recur more frequently, and, therefore, to be of smaller magnitude than later, when the interval between them, and correspondingly their size, tend to increase. The first contraction of a series, thus, is usually considerably larger than those immediately succeeding it. Occasionally, however, they increase gradually in size from the outset in staircase fashion (fig. 5). The relaxation following the contractions is quite complete, unless they occur so frequently that the interval between them does not permit full extension. This is frequently the case at the beginning of a period of stimulation.

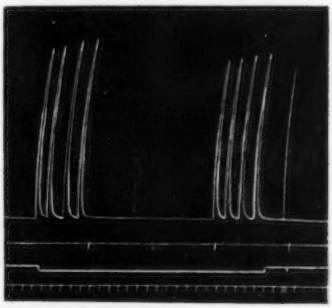

Fig. 5. Non-poisoned apex of ventricle of tortoise. Frequencies: 2.5; 10; 2.5 per second; changes indicated by downstrokes in upper most signal line. 320 Z units.

A moderate increase in frequency of stimulation, while these rhythmic contractions are in progress, prolongs the interval between them and

usually augments their magnitude. The change in frequency required to produce this effect, for the same strength of stimulus (320 Z units), varies with different preparations. The upper limit for the occurrence of the reaction was an increase to 20 per second, but it was well marked in a number of experiments with an elevation to only 5 per second. In two experiments, the threshold was determined, and the frequency required to slow the rhythm was somewhat higher for the more excitable preparation. With a corresponding decrease in frequency, the reverse change takes place. The contractions occur at more brief intervals and they are usually diminished in magnitude.

A somewhat greater increase in frequency than that which suffices to slow the rhythm leads to complete cessation of the contractions. For a given strength of stimulus (320 Z units), the highest frequency required to produce this effect was 30 per second, and the lowest was 10 per second. In the two experiments in which the threshold was taken, it was necessary to employ a somewhat higher frequency for the more excitable tissue. The change in reaction takes place immediately. It is not preceded by contraction (fig. 5). During the period of inactivity, a slight tonic shortening may develop, but at times the relaxation is so complete that the tracing gives no indication of contraction (fig. 5). The relaxation level thus may be considerably lower than that during the progress of the contractions. A corresponding fall in frequency leads to the reappearance of the rhythmic contractions, often preceded by a prolonged latent period. The greatest delay occurred with a decrease from 30 to 10 per second. It amounted to six seconds, and it was accompanied by a partial relaxation from the slight tonic contraction, which had been set up by the higher frequency. This period of quiescence, moreover, favors a return of the preparation to the condition preceding stimulation, as indicated by the character of the contractions which occur when the frequency is lowered. If, at the beginning of stimulation, they rise in staircase fashion, they increase in the same manner when the frequency is diminished. If the contractions are reduced in extent as a result of rapid recurrence, on the other hand, the first following the intercalated period of relaxation is larger than those preceding or succeeding it.

A few observations were made in which the apex of the ventricle was stimulated at the outset with frequencies too high to maintain rhythmicity. Interruptions at the rate of 7.5 per second, with intensities (320 and 370 Z units) considerably above threshold value, resulted in a few introductory contractions well limited to the beginning of the period of stimulation. Frequencies of 15 per second and above, with corresponding intensities, evoked only a single contraction, followed by quite complete relaxation In one observation, a distinct tonic shortening developed after the contraction produced by 40 per second, and this was reduced somewhat on

diminishing the frequency by half. Cessation of stimulation, while the muscle was relaxed thus, was not attended by contraction.

Systematic experiments to test the effect of variation in intensity of stimulation on these results were not undertaken, but the data obtained indicated that intensity of stimulus, provided that it was well above threshold, was not an important factor in producing the changes in reaction. In one experiment, for example, an initial contraction only was produced by a frequency of 15 per second and an intensity of 455 Z units. Decreasing the former to 12.5 per second and the latter to 390 Z units resulted in only two introductory contractions. When the intensity was increased to 570 Z units, furthermore, a frequency of 17.5 per second led to complete cessation of contractions in progress in response to the lower frequency of

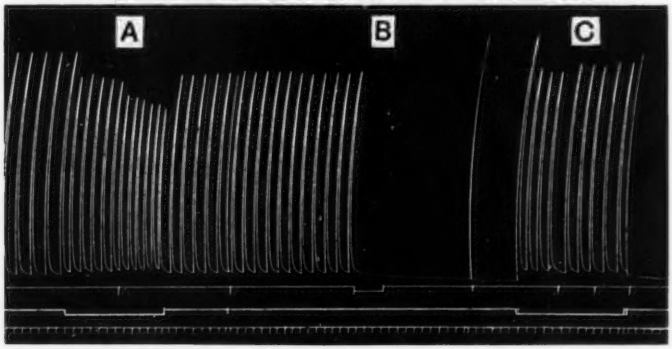

Fig. 6. Atropinized apex of ventricle of tortoise. A: 690 per second; intensities, 320 and 415 Z units; change indicated by downstroke in uppermost signal line. B: Effect of dropping on 2 per cent pilocarpine hydrochloride in Ringer solution. C: Frequencies: 160, 400, 560 per second; changes indicated by downstrokes in uppermost signal line; 415 Z units.

1.25. In another experiment, contractions in progress as a result of stimulation with a frequency of 7.5 per second ceased with an elevation to 12.5, the intensity being 145 Z units, and later, when the strength was increased to 205 Z units, they disappeared at a frequency of 15 per second.

The application of 0.2 per cent atropine sulphate in Ringer solution to the apex of the ventricle increases greatly its ability to respond with rhythmic contractions to relatively high frequencies of stimulation. A concentration of 0.1 per cent of the drug, however, is quite ineffective. When the oxygenated atropine-Ringer solution was siphoned off from the preparation, which yielded figures 5, 6 and 7, the tissue responded with strong rhythmic contractions to frequencies as high as 480 per second. The fifth period of stimulation after atropinization established spontaneous rhythmicity. A frequency of 690 per second thereafter only accelerated the rhythm and

reduced the height of the contractions, as shown in figure 6, A. Such reactions are fairly typical for atropinized preparations. For intensities about 320 Z units, they contract rhythmically, as a rule, in response to the highest frequencies employed, viz., up to 720 or thereabouts per second. They show, furthermore, a marked tendency to spontaneous rhythmicity, especially after having been set into activity by electrical stimulation. Two preparations exhibited rhythmic contractions for a brief period before they were subjected to the action of induction shocks.

As in the case of the non-atropinized preparation, the size of the contractions is directly proportional to the interval between them, but a decrease in stimulus interval does not always bring about a slowing of the rhythm. Increasing the intensity of stimulation, the frequency remaining constant, results regularly in a shortening of the interval between contractions and a corresponding diminution in their magnitude (fig. 6, A), whether the preparation is spontaneously active or not. The inverse relation of frequency of stimulation to frequency of contraction, however, usually does not hold within the range of 15 to 240 per second, for the usual intensity employed (320 Z units). A frequency of 80, for example, may evoke a more rapid rhythm than one of 40, and the latter, more frequent contractions than one of 10 per second. In one observation, a distinct pause in the rhythm, followed by a contraction stronger than its predecessors, and successors, attended a decrease from 240 to 80 per second. The interval between the contractions produced by the higher frequency was slightly less than that for the lower. A further decrease to 40 per second, in the same period of stimulation, was attended by a similar series of events. When a frequency above 240 per second is raised further, however, the rhythm is slowed decidedly, as a rule, and the slowing is preceded by a prolonged pause. The interval between contractions was increased also for elevations in frequency between the limits of 2.5 to 15 per second. One experiment was an exception to this description. Parke-Davis "adrenalin" (1:10,000 in Ringer solution) had been dropped on the tissue previous to the application of atropine, and the intensity was nearly 600 Z units. Under these conditions, elevations in frequency between the limits of 2.5 and 240 per second resulted only in slowing of the rhythm. An augmentation from 40 to 400 per second led to complete cessation of contractions.

Certain other differences in the reaction of atropinized and non-atropinized preparations were observed. In one observation, a contraction occurred in transition from 400 to 240 per second, which was separated from its predecessor by only 4 seconds. The contractions in progress before the change, however, were about 17 seconds apart, their frequency gradually decreasing. Those following the transition were about 10 seconds apart, their frequency gradually increasing. The contraction of transition, therefore, was considerably smaller than its fellows. A similar contraction

occurred in a following period of stimulation, when the frequency of interruption was increased from 2.5 to 10 per second. In this case the preceding contractions were separated by an average interval of six seconds, and those succeeding by intervals of eight seconds. A prolonged latent period preceding contraction, on lowering the frequency from an apparently ineffective to an excitatory value, was not observed for the atropinized preparation.

In two experiments the effect of atropine on the threshold was determined. Immediately after taking the tracing represented in figure 5, the threshold position of the secondary coil for the break shock was 9.5 cm., corresponding to 295 Z units, and that for the make was 10.2 cm. The tissue was covered then with oxygenated 0.2 per cent atropine sulphate in Ringer solution for a period of 15 minutes, and the threshold was tested again. A somewhat stronger break shock (secondary at 9.2 cm., 310 Z units) was required than before. The threshold for the make, however, was lowered somewhat, being reached at a secondary position of 11.0 cm. In the second experiment the threshold position for both make and break at first was practically the same, viz., 11.0 cm. (break intensity, 205 Z units). Atropinization lowered the threshold for the make to 11.5 cm., and that for the break to 12.5 cm. (115 Z units).

It may be mentioned incidentally that the atria appear to be more sensitive to the action of atropine than the apex of the ventricle. Into one tortoise, 15 cc. of 0.2 per cent atropine sulphate in Ringer solution had been injected intravenously five hours before excision of the heart. Stimulation of the atria thereafter, by the method described for the apex of the ventricle, caused only a decrease in the interval between contractions and a corresponding diminution in their size. This result was obtained for frequencies varying from 40 to 480 per second, the position of the secondary coil being 7 cm. (415 Z units). The effect was more pronounced for frequencies of 80 and 160 than for 40 per second, and for the latter, more than for the higher frequencies of 320 and 480. The reaction in this respect resembled that most often seen in the atropinized apex of the ventricle. This structure, on the contrary, was apparently unaffected by the injection of the drug. The relatively low frequency of 7.5 per second (320 Z units) was sufficient to cause cessation of the rhythm in progress in response to 2.5 per second.

Pilocarpine counteracts the effects of atropine. Concentrations of 0.5 and 1.0 per cent of the hydrochloride are quite ineffective, but a 2.0 per cent solution manifests its action immediately. For the development of the full influence of the drug, however, the solution must be left in contact with the tissue for a considerable period. As shown in figure 6, B, dropping on the 2.0 per cent solution results at once in cessation of the spontaneous contractions and gradual relaxation of the muscle. That its full

effects are not established immediately, however, is shown by the following period of stimulation (fig. 6, C), in which frequencies as high as 560 per second evoked rhythmic contractions. The application or ordinary Ringer solution does not lead to prolonged cessation of the spontaneous contractions, and sodium chloride solution acidified with hydrochloric acid increases both the tonus and the frequency of contractions of the muscle.

Immediately after the observation shown in figure 6, C, the preparation was covered for 21 minutes with 2.0 per cent pilocarpine hydrochloride in oxygenated Ringer solution. About 25 minutes later, a frequency of 1.25 per second (530 Z units) produced a regular rhythm of contractions, but these ceased entirely when the frequency was increased to 2.5, as shown in figure 7. It is seen that the relaxation in response to the more frequent stimuli was quite complete. Lowering the frequency to its preceding value, as shown in the figure, led to the reappearance of the contractions after a latent period of about three seconds. The relation of frequency of stimulation to effect produced, therefore, was quite similar to that observed for the unpoisoned preparation, except that in this experiment, the frequency required to cause cessation of contraction was less (cf. fig. 5).

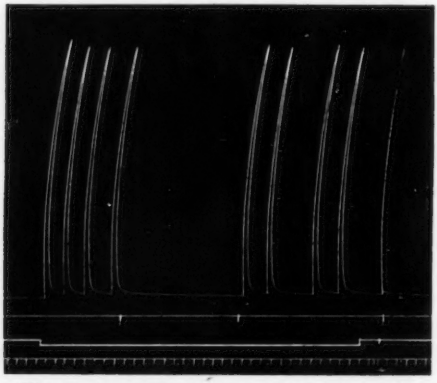

Fig. 7. Apex of ventricle of tortoise. Pilocarpine after atropine. Frequencies: 1.25; 2.5; 1.25 per second; changes indicated by downstrokes in uppermost signal line. 530 Z units.

Soon after the tracing represented in figure 7 was taken, the tissue was subjected to a series of periods of stimulation, with frequencies ranging from 3.75 to 720 per second. Each of these evoked a single initial contraction, followed during stimulation by almost complete relaxation. For frequencies up to 400 (520 Z units), however, a slight tonic shortening persisted till cessation of faradization. This was scarcely detectable for the lowest frequency, but it increased progressively as the stimulus interval was reduced, reaching a maximum at 80 per second. For frequencies above this, the tonic shortening became progressively less pronounced, being hardly noticeable at 400. Succeeding periods of stimulation at 720 per second (635 Z units) failed to produce initial contractions, if the intervals between faradization were sufficiently brief. With stimulation lasting 10 seconds, an initial contraction was produced by each of two succes-

sive periods separated by an interval of four seconds. A third period of stimulation, following the second after 1.7 seconds, failed to evoke a contraction. Likewise a fourth period, separated from its predecessor by three seconds, was ineffective. A fifth period, however, succeeding the fourth after four seconds, produced a contraction. No clear indication that a tonic contraction was caused by this frequency was given by the tracing. The relaxation for any of the frequencies, furthermore, was not followed by after-contraction on cessation of faradization.

Pilocarpine (2.0 per cent) raises the threshold for faradic stimuli. Covering a preparation with non-oxygenated pilocarpine-Ringer solution for an hour led to an elevation for the break shock from approximately 200 to 500 Z units. In a second experiment, the tissue was left in an oxygenated pilocarpine-Ringer bath for 21 minutes, a procedure which raised the threshold for the break from about 310 to 550 Z units. That for the make rose correspondingly. In both cases, however, the threshold fell gradually after the solution of the drug had been drained off. The contractions of the second preparation, for a period of 15 minutes after treatment with pilocarpine and preceding the taking of the tracing represented in figure 7, were not of an all-or-none character. They first appeared, as the intensity was gradually increased, for the break shock, and as the stimuli were made still stronger, the contractions increased in magnitude. That for the break, for a given position of the secondary coil, was greater than that for the make.

In the experiment from which figure 7 was taken, and after the successive periods of stimulation with increasing frequencies referred to above, the preparation was covered again with the oxygenated atropine-Ringer bath for 15 minutes. As a result, the threshold for the break shock fell from 380 to 280 Z units and that for the make was lowered correspondingly. A considerable degree of tonus was manifested, moreover, by relaxation after contraction to a level below that of the resting preparation. At first, a frequency of 5 per second evoked a much slower rhythm than one of 1.25, and an increase from 5 to 10 per second, after producing a contraction of transition, led to complete cessation of the rhythm. The tonus rose in intervals between the contractions set up by 5 per second slightly above that of the resting preparation, and somewhat more so in response to 10 per second. As a result of the second period of prolonged stimulation, however, a spontaneous rhythm was established. While this was in progress, a period of stimulation at 160 per second (secondary at 9 cm., 320 Z units) had an inhibitory effect. It produced first an initial contraction, smaller than those preceding it, and this was followed by incomplete relaxation. With continued faradization, the tonus rose gradually, but contractions failed to appear. At the end of the period, the tonus decreased decidedly, though remaining considerably higher than that of

the spontaneously rhythmic preparation, and contractions failed to appear for a period of a minute. Thereafter frequencies as high as 720 per second only accelerated the rhythm, with positions of the secondary varying from 8 to 3 cm. The application of pilocarpine, however, resulted in immediate cessation of the contraction and gradual relaxation of the muscle.

In one experiment, the effect of Parke-Davis "adrenalin," freshly diluted (1:10,000) in Ringer solution, was tested on the threshold of a very inexcitable preparation. Before dropping on the solution, the threshold for the break shock was above 1800 Z units, but immediately afterwards it had fallen to 495 Z units.

Results from smooth muscle. The experiments on direct stimulation of smooth muscle were few in number, but on the whole the results were in agreement with those obtained from the other varieties. The siphon muscle of the clam was used in the preliminary experiments, and neither the frequency nor the intensity of stimulation was accurately determined. The former ranged probably between 3 and 40 per second, however, and the latter probably amounted to several hundred Z units. When the highest frequencies were employed at the beginning of a period of stimulation, a relatively weak initial tetanus usually occurred, gradual relaxation taking place during faradization. A change to a low frequency, under these conditions, led at once to strong and well-maintained contraction. The extent of this contraction was often several times that of the preceding contraction produced by the higher frequency. In one period of faradization, a return to the more frequent stimuli resulted in a transient increase in the shortening, followed by gradual relaxation. The fall in the curve was not appreciably accelerated on cessation of stimulation. This slow relaxation of the muscle rendered it somewhat unsuitable for experimentation.

Only one of the Magnus preparations reacted well to stimulation. It was taken from the circular coat of the duodenum of a cat anesthetized with urethane. It showed a tendency to become inexcitable after prolonged faradization, however, and for that reason the periods of stimulation were separated by intervals of 10 to 15 minutes, in which the preparation was covered with oxygenated Ringer solution near 37°C. For most of the observations, the position of the secondary coil was 4 cm., corresponding to a strength of break shock of 530 Z units. Frequencies of 40 and 80 per second caused slowly developing, smooth contraction. In one of the first observations, 160 shocks per second, though causing only slight contraction at the beginning of the period of stimulation, apparently changed the rising phase of the shortening produced by 40 per second into an evenly maintained contraction, questionably resembling tonus somewhat. A further increase to 320 per second caused relaxation, and a succeeding decrease to 40 produced again contraction. In later periods

of faradization, 320 per second caused this change to apparently tonic contraction, though having no evident effect at the beginning of stimulation. A frequency of 480 was required then to bring about relaxation. No evidence of an initial contraction effect was obtained in the course of the experiment.

The lowermost two centimeters of the esophagus of the cat, when arranged for recording the contractions of the longitudinal coat, relaxes in response to a relatively high frequency of stimulation, provided that it has been thrown previously into contraction by a lower frequency. In the first experiment this structure was taken from a cat anesthetized with urethane. It was arranged at room temperature, and stimulated with a break-shock intensity of 1,170 Z units. A frequency of 100 per second produced a relatively weak and poorly maintained contraction, the rhythmic relaxation being almost complete in the course of a minute. Lowering the frequency to 20 per second then caused strong contraction, but immediate and quite complete relaxation took place with a succeeding increase to 100 per second. This result was obtained repeatedly. In case stimulation at 20 per second was long continued, gradual relaxation took place, which was rhythmic in character. A succeeding increase to 100 then not only caused relaxation of the muscle, but established conditions permitting strong contraction on a following decrease to 20. The extent of this contraction was much greater than that immediately preceding the elevation in frequency. Similar results were obtained in a second period of stimulation with alternations between 80 and 20 per second. The contraction produced at the beginning of the period by 80, however, was stronger and better maintained than the corresponding contraction produced by 100 in the first observation. In the final period of stimulation, when the preparation was in poor condition, a frequency of 5 per second was apparently without effect, whereas an increase to 15 caused strong contraction.

In a second experiment, the portion of the gullet was taken from a cat decerebrated by pithing under ether anesthesia, time having been given for the animal to exhale most of the drug. The preparation was arranged as described under experimental methods. At first the tonus was low, and strong rhythmic contractions of prolonged duration were in progress. Under these conditions, the tube was subjected to faradization, the position of the secondary coil being 10 cm. (265 Z units). The tracing obtained is represented in figure 8. It is seen that a frequency of 240 caused pronounced, rhythmic and fairly well-maintained contraction (fig. 8, A). An increase to 400 per second, however, led to almost complete relaxation. Decreasing the frequency then to 160 produced a contraction, which was as strong as that evoked by 240 and decidedly better maintained. A succeeding elevation to 400 had the same effect as before, and the course

of the relaxation was interrupted by strong contraction when the frequency was lowered to 80. Promptness of occurrence is a marked feature of the contractions taking place on lengthening the stimulus interval as well as their great magnitude. A period of rest of about two minutes' duration resulted in a marked change in reaction. A frequency of 400 per second

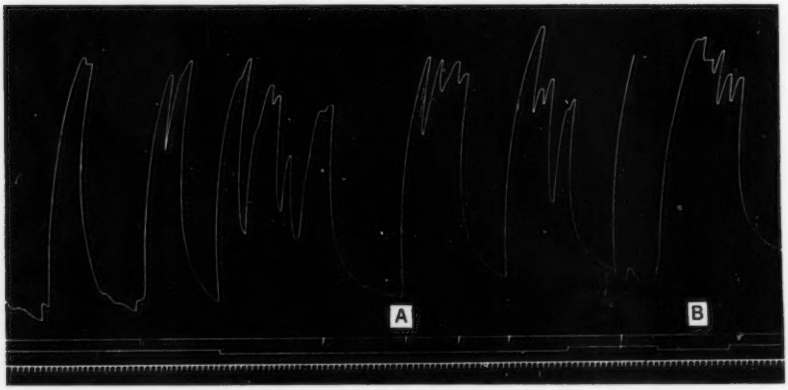

Fig. 8. Lower end of esophagus of cat. A: Frequencies: 240; 400; 160; 400; 80 per second; changes indicated by downstrokes in uppermost signal line; 265 Z units. B: 400 per second; 265 Z units.

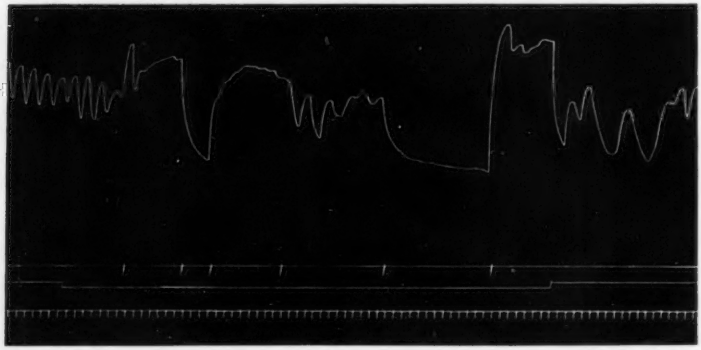

Fig. 9. Lower end of esophagus of cat. Frequencies: 400; 240; 400; 80; 320; 400; 80 per second; changes indicated by downstrokes in uppermost signal line. 265 Z units.

then, as shown in figure 8, B, had the reverse effect of producing strong and well-maintained contraction.

After the tracing reproduced in figure 8 was taken, the preparation was covered over with oxygenated Ringer solution for a few minutes, and dur-

ing this period, the tonus of the muscle increased greatly, and its rhythmic contractions became more frequent, as shown in figure 9. Under these conditions, likewise, the predominant effect of the lower frequencies was to cause increased contraction, whereas that of the higher frequencies was inhibitory. It is to be observed, however, that a frequency of 400 per second (secondary at 10 cm., 265 Z units) was at first without decided effect on the mechanical changes of the muscle. Loss of tonus and disappearance of rhythmic contractions, in response to this frequency, became well marked only after the preparation had been subjected to the action of stimuli separated by wider intervals. It may be pointed out in addition that though the final motor effect of 80 per second was greater than any preceding it, spontaneous rhythmicity and tonus made their appearance without delay on cessation of stimulation.

Experiments performed at Cambridge. *Experimental methods.* The experiments at Cambridge were carried out in much the same way as those described above, with the exception that the tissue was stimulated through two pairs of electrodes. Preparations of the gastrocnemius and sartorius of Rana temporaria, and of the apex of the ventricle of Testudo iberia were subjected to faradization. Curarization of the frogs was effected, after destruction of the brain, by injecting doses varying from 1 cc. of 0.2 per cent to 0.5 cc. of 1 per cent curare in Ringer solution into the dorsal lymph sac, the animals being considered curarized when pinching the sciatic failed to cause contraction of the gastrocnemius. Silver wires were used as electrodes for the higher frequencies, and these were thrust into the preparation usually near its fixed end. The low frequency electrodes, which were fine copper wires, pierced the tissue within 3 or 4 mm. of the other pair or at the opposite end of the preparation. For the gastrocnemius, the silver wires were placed always at the knee end, the copper electrodes being inserted a few millimeters above the tendon end. The testing induction shocks, delivered by the latter, were usually strong enough to cause maximal twitches in the case of the curarized sartorius, but for the apex of the ventricle they were near threshold value. Each pair of electrodes was connected by fine copper wires to the binding posts of the moist chamber, and from these directly to the secondary of an inductorium.

The primary circuit for the higher frequency was made and broken by a rotary interrupter described by Adrian and Olmsted (20), the duration of closure being 50 per cent. A decrease in frequency during a period of stimulation could be brought about with this apparatus in an interval of two seconds. To change the direction of the relatively high frequency induction shocks, a hand operated commutator placed either in the primary or secondary circuit was employed. For the lower frequency, an interrupter designed by Keith Lucas and described by Adrian (21) was

used. The primary circuit was made and broken at first by a contact operated by one of the cam wheels (D_2 in Adrian's figure) driven by the shaft of the motor, and the desired frequency was obtained by means of a series of cam wheels (E in Adrian's figure) driven by the worm reduction gear. Later, however, this series (E) alone served as interrupter. The duration of each revolution of the motor of the Lucas apparatus was about 32.3σ, and when the current was interrupted by the shaft-driven cam wheel, the interval between the make and the succeeding break shock was about 15σ. When the series (E) interrupted the current, however, this interval was about 30σ. In all cases, the testing shocks were delivered in make-break couples at intervals of 1.03 seconds.

Results. With the curarized gastrocnemius and sartorius, no evidence of suppression of the periodic contractions by relatively high frequencies of

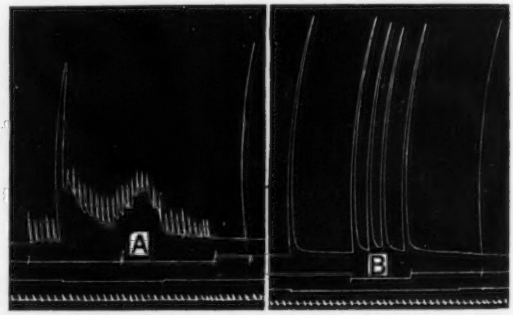

Fig. 10. A: Curarized sartorius of Rana temporaria. Cu electrodes within 4 mm. of Ag. Uppermost signal: 1.03 per second. Middle signal: 160 per second to upstroke in uppermost signal line; then 32. Time in 1.2 second intervals. B: Apex of ventricle of Testudo iberia. Uppermost signal: 1.03 per second. Middle signal: 72 per second. Time in 6 second intervals.

stimulation was obtained. This was true for the sartorius whether the two pairs of electrodes were separated from one another by only 3 or 4 mm., or whether they were at opposite ends of the preparation. An example of such an observation, with the former arrangement, is given in figure 10, A. That an initial tetanus was produced is shown by the rise in the contraction level occurring on lowering the frequency from 160 to 32 per second. In other observations on the same muscle, this increase in the shortening was more pronounced than that shown in the figure, and there was some indication that the muscle continued to contract for some time after the frequency had been lowered. The general form of the tetanic curve was apparently not affected decidedly by the occurrence of the periodic contractions. In the case of the non-curarized gastrocnemius, however, more or less complete suppression of the periodic contractions was

often brought about by the higher frequencies. It was always preceded by the usual initial tetanus effect.

The following incidental observations on the curarized sartorius may be mentioned. In one instance, a decrease to 32 per second, after the production of an initial contraction by 120, resulted in a gradual rise in the contraction level together with the appearance of irregularities resembling rhythmic contractions somewhat. A return to 120 led to cessation of the irregularities and almost complete relaxation of the muscle. In a number of experiments, furthermore, in which the muscle had failed almost completely to respond to tetanizing faradization, as a result of preceding periods of stimulation, changing the position of the electrodes a few millimeters resulted in pronounced contraction. One instance of reversal was observed, similar to those described on p. 263 for the gastrocnemius. The sartorius had been taken from a frog in poor condition, and 120 per second excited it more effectively to maintained contraction than 32, though the shortening in response to the former was of relatively small magnitude.

The apex of the ventricle of Testudo iberia, when freshly prepared and taken from active tortoises, usually responds to the different frequencies in much the same manner as that described for the similar preparation on pp. 267–9. Sending in shocks, near the fixed end of the structure, at a frequency sufficiently high to produce the initial contraction effect does not prevent the occurrence of a series of rhythmic contractions in response to a lower frequency applied at the opposite end. There appears to be no fall in excitability at the low frequency electrodes, and no decided change in the frequency of occurrence nor in the magnitude of the contractions set up by them. Figure 10, B, illustrates the effectiveness of low frequency stimulation, while the higher frequency is still being applied. In the one experiment in which the copper electrodes were placed within 3 mm. of the silver ones, moreover, the results were quite similar to those shown in the figure. The apex of the ventricle of Testudo iberia, however, shows a tendency to spontaneous rhythmicity without the application of drugs —a characteristic distinguishing it from the preparation from fresh water tortoises of North America. Often after a few periods of stimulation giving the usual results, moreover, it responds with rhythmic contractions to quite high frequencies. Incidentally it may be added that quickly changing the direction of induction shocks, of sufficient frequency to produce the initial contraction effect, apparently evokes no change in the reaction of the muscle. It remains quite completely relaxed, and there is usually no obvious change in the slight tonic shortening frequently observable after the occurrence of the initial contraction.

Discussion. The action of atropine in counteracting the effects of the higher frequencies on the gastrocnemius and the apex of the ventricle, and the abolition of the influence of this drug, in the case of the latter, by pilo-

carpine, are facts suggesting that these frequencies bring about a true inhibition. Further evidence that their action is inhibitory is given by the reaction of the lower end of the esophagus, for in this structure they effected a cessation of activity spontaneously in progress. It might be presumed that the contraction produced by the lower frequencies, in this case, so deranged the functioning of the muscle that it was unable to contract spontaneously thereafter. On this view the relaxation would be a condition of rest of the muscle, because of a supposed ineffectiveness of the higher frequency. That the muscle retains its ability to contract spontaneously, however, is shown in figure 9, by the fact that, though the shortening in response to the final frequency was greater than any preceding it, spontaneous activity was resumed immediately on cessation of stimulation.

It is obvious that fatigue of the muscle as a whole, in the sense of a diminution in its ability to do work, is not the cause of the reaction. It may be suggested, therefore, that the more rapid fall of tetanus curves of directly stimulated skeletal muscle, with increase in the frequency of the stimuli, may be the result of a condition established about the electrodes, rather than the more rapid exhaustion of the muscle (22) (4). The failure of after-contraction to occur on cessation of stimulation with the higher frequencies, and the failure of contraction of the apex of the ventricle to take place on changing the direction of the stimuli indicate, furthermore, that the effects of the more frequent shocks are not entirely the result of polarization.

It is quite certain that inhibitory nerves, or a special inhibitory receptive substance (23), do not play an important part in evoking the relaxation. In skeletal muscle (24) and the apex of the ventricle (25) (26) (27) (28) there is no definite evidence for their existence. Kronecker (26) detected no change in the excitability of the ventricle of Testudo graeca during vagal arrest of the heart, and one of us (V.) has found that the reactions of the ventricle of Testudo iberia to the different frequencies of direct stimulation are not affected to a marked degree by faradization of the vagus. During vagus standstill, relatively low frequencies evoke rhythmic contractions, whereas higher frequencies give the initial contraction effect. In numerous observations on fresh water tortoises of North America, moreover, one of us (V.) has detected no indication of negative inotropic effects on the ventricle, the contractions being recorded by a heart lever, when simultaneously the beats of the atria were scarcely registrable because of vagal inhibition. The inhibitory effects of stimulation of the vagus and splanchnic nerves on the lower end of the esophagus, furthermore, may be considered of the nature of Wedensky inhibition (1).

It seems most probable that the inhibitory reaction is the result of an excessively high frequency of stimulation. The slow recovery of the gastrocnemius and the apex of the ventricle from the effects of the higher

frequencies, observed at times, suggests that stimulation fatigue is an important factor in the reaction. The work of Gotch (29) on nerve indicates that this factor may either cause a diminution in the magnitude of the propagated disturbance or prevent it from appearing. It might be suggested also that a reduction in the magnitude of the disturbances in muscle might occur as a result of each travelling in the relative refractory state left by its predecessor (30). If propagated disturbances are set up at all during inhibition of cardiac and skeletal muscle by the higher frequencies, however, the localization of the effect shows that they must be conducted with a decrement. It might be supposed on the basis of Engelmann's experiments on the ureter (13) (14), that conduction with a decrement would occur. In the case of the lower end of the esophagus, however, the action of the intramuscular nerves was not excluded, and it is possible that these were stimulated at a frequency sufficiently high to produce inhibiton (1).

In view of the results reported herein, it may be suggested, in addition to the considerations presented in a preceding paper (1), that the factor of stimulation fatigue may be of importance in inhibition of smooth muscle by nervous action. It seems probable that such is the case inasmuch as the inhibitory effects of the higher frequencies of direct stimulation of the lower end of the esophagus became manifest only after this structure had been subjected to faradization for a time. In the case of inhibition by the vagus, the stimulation fatigue would be brought about by the action of propagated disturbances on that part of the muscle cell which excites its effector mechanism to activity (31).

In reference to inhibition of the heart, it is probable that the inhibitory nerves act on the rhythm-producing centers, such as the sino-auricular node, in such a way as to reduce the magnitude of the physico-chemical changes within them to subthreshold value for the structures which these disturbances normally excite to rhythmic activity. This reduction in magnitude might be brought about by stimulation fatigue, or by an increase in the frequency of occurrence of the disturbances, or by both factors acting together. The former factor, of course, might be considered sufficient to abolish them completely.

The inhibitory effects produced by the higher frequencies in denervated and curarized skeletal muscle indicate that Wedensky inhibition may be the result of a condition established beyond the terminations of the nerves, which frees the muscle from excitation. This view becomes the more probable in consideration of the fact that the initial contraction on indirect stimulation is usually a brief tetanus rather than a single twitch, even in fatigued, weakly curarized, nicotinized or etherized preparations (32). Frequencies of stimulation much too low to permit reduction in the magnitude of the nerve impulse as a result of the relative refractory state left by

its predecessor (33), furthermore, may produce the Wedensky effect (34) (35), and the analogous phenomenon observed on stimulation of the nerve centrally of a locally narcotized region (36). It may be suggested, therefore, that a condition similar to that produced by the higher frequencies of direct stimulation develops in the junction between the nerve terminations and the protoplasm of the neural region of the muscle fiber, or possibly within the latter, as a result of the action of a relatively high frequency of nerve impulses.

In reference to the rhythmicity of the gastrocnemius, the fact that it appears only after the muscle has become somewhat fatigued suggests that fatigue and recovery of the individual muscle fibers, with consequent rise and fall[2] respectively in their threshold to faradic stimulation (37) are important factors in its causation. It shows some resemblance to rhythmicity of the heart, and the effect of atropine in facilitating the occurrence of rhythmic contractions of both the gastrocnemius and the apex of the ventricle, in response to relatively high frequencies of stimulation, suggests that in both cases the rhythmicity is of the same fundamental nature. On the same grounds, a similar conclusion might be drawn in reference to inhibition in the two types of muscle. The fact that atropine has its effects on curarized gastrocnemii shows that its seat of action is distal to the nervous mechanism. It is probable, therefore, that its action in preventing inhibition of the heart by the vagi is exercised beyond the limits of the peripheral neurones. Pilocarpine would be expected to have the same seat of action, inasmuch as it counteracts the effects of atropine on the apex of the ventricle. The tendency of atropine to raise the excitability of this structure, and of pilocarpine to lower it, probably has direct bearing on the effects of these drugs on the response of the muscle to the different frequencies of stimulation.

SUMMARY AND CONCLUSIONS

1. Relatively high frequencies of direct stimulation of skeletal, cardiac and smooth muscle have inhibitory effects, whereas relatively low frequencies are motor (figs. 1, 2, 3, 5, 8 and 9).

2. Atropine counteracts the inhibitory effects of the higher frequencies on the apex of the ventricle of the tortoise (fig. 6) and the curarized (fig. 4) or non-curarized gastrocnemius of the frog. For the gastrocnemius, this action of the drug is shown particularly by the persistence of rhythmicity during stimulation with relatively high frequencies. In the case of the apex of the ventricle, pilocarpine abolishes the action of atropine (fig. 7).

3. The effects of the higher frequencies are closely limited to the elec-

[2] The word, "lower," in 1.40, p. 226, of the preliminary report on this investigation should be "raise."

trodes in the case of skeletal and cardiac muscle (fig. 10), but the character of the reaction of spontaneously active smooth muscle (fig. 9) indicates that nearly all of its cells are affected.

4. It is suggested that stimulation fatigue, possibly connected with a diminution in the magnitude of the propagated disturbances and their conduction with a decrement, may be responsible for the occurrence of the inhibitory reaction. It is suggested, furthermore, that these factors may be involved in inhibition of the heart and of smooth muscle by nervous action. This phenomenon in these structures, therefore, is probably related, in part at least, to Wedensky inhibition.

5. Infrequently the higher frequencies may be more effective in producing contraction in skeletal muscle in poor condition than the lower.

6. Atropine and pilocarpine act beyond the limits of nerve, and probably, therefore, directly on muscle.

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SOME OBSERVATIONS ON DECEREBRATE RIGIDITY AND PLASTIC TONUS AFTER REMOVAL OF THE LUMBAR SYMPATHETICS

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On the anatomical side considerable evidence has gradually been accumulating which indicates that skeletal muscle may receive a sympathetic as well as a somatic innervation. The original observation seems to have been that of Tchiriev but confirmation has been afforded by the work of Boeke, de Barenne and Agduhr. A further development has been the apparent demonstration by Perroncito, Kulschitsky and Hunter that the medullated and non-medullated nerve fibers never terminate in the same muscle fiber. Hunter, impressed by the old work of Ranvier describing two types of muscle fibers, believed that the sympathetic nerve terminates in thin muscle fibers rich in protoplasm while the medullated somatic nerve terminates in the thick, pale muscle fibers. A complete review of this literature may be found in the papers of Kavenel, Pollock and Davis (1925) and those of Hunter (1925)

On the physiological side Mosso (1904) first suggested that the sympathetic nervous system might govern muscle tonus, a point of view supported by the work of De Boer (1915) and de Barenne (1916) although the latter, three years later, reversed his position on the matter. Langelaan (1915) at about the same time advanced the idea that contractile tonus was mediated by the somatic fibers while plastic tonus was taken care of by the sympathetics. During the next eight or nine years, working almost exclusively on frogs and cats, a number of investigators failed to find any relation between the sympathetics and muscle tonus. Only two exceptions seem to have occurred. Manmarry (1922) found that extirpation of the labyrinth on one side and the sympathetic chain on the opposite produced loss of muscle tonus only on the side of the sympathectomy, and Rogowitsch, quoted by von Rijnberk (1916), noted that after cutting the facial nerve, stimulation of the annulus of Vieussens still gave some motor effects.

This was about the situation until 1924 when the work of Hunter and Royle began to appear. These authors, building on the anatomical work already mentioned, the physiological researches of Sherrington and the

suggestions of Langelaan, developed the following conception of muscle tonus. Contractile tonus imposes posture as a result of the shortening of appropriate muscle groups. Plastic tonus maintains this posture, once it has been attained, by fixing the length of the muscles exhibiting the contractile tone. The reflex nervous mechanism for contractile tonus consists of afferent proprioceptive impulses, an arc at least as high as the pons, probably through Deiter's nucleus, and a discharge through the vestibulospinal tract and the somatic motor nerves to the thick contractile muscle fibers. The mechanism for plastic tonus consists of the same proprioceptive afferents, an arc in the formato reticularis of the pons, and a discharge through the ponto-spinal tracts, the white rami and the post ganglionic sympathetic fibers to the slender plastic tonus muscle fibers.

To test the physiological side of this hypothesis, Hunter and Royle used the method first employed by Cobb (1918). This consists in the removal of the sympathetic innervation to some group of muscles and then decerebration. If in the state of decerebrate rigidity plastic tonus still appears in the sympathectomized muscles it is obvious that it is not dependent on the sympathetic innervation. Cats have almost always been used in such experiments. Hunter and Royle did not have much success either with rabbits, cats or dogs, but in fowls and goats their results were positive. In the fowl the wing on the operated side was held at a lower level which was interpreted as the loss of plastic tonus or the ability to fix the muscles at a given length of contraction. In the goat, after resection of the lumbar sympathetic rami and subsequent decerebration, the leg on the operated side rather quickly fell from the temporary extension induced by putting the animal on its back. This limb was much easier pressed down than the one on the unoperated side. These effects were interpreted as being due to loss of plastic tone. These results in their animals have apparently been confirmed by Royle on some human cases in which he has done sympathetic ramisectomies for the relief of spastic paralyses.

Experimental. The failure of other workers to show a loss of plastic tone after sympathectomy and decerebration has been attributed by Hunter and Royle to two factors, the selection of an unsuitable animal and neglect to wait a long enough time after removal of the sympathetics before making the test. In view of the first criticism it would seem desirable to test the effect of the sympathetics on as many laboratory animals as possible. We have therefore used dogs, on which few experiments have been made. In regard to the necessity of a considerable interval between the time of sympathectomy and the subsequent test for loss of plastic tone, it is difficult to see why the absence of something should appear only some months after it is gone. We have, however, allowed considerable time to intervene in order to meet this criticism of the Australian investigators.

We wish to report briefly the results of removing the sympathetic innervation to the right hind leg of dogs. In nine experiments the attempt was made to cut all the mesial branches of the sympathetic chain on the right side from the second to the sixth lumbar. Surgical approach was made from the dorsal lumbar region. In three other experiments the entire chain was removed in the lumbar region, through a mid-line abdominal incision. It is believed (Hunter, 1925) that the sympathetic nerves supplying the muscles of the lower limb arise from the lumbar ganglia and that the preganglionic contribution ceases at the level of the second lumbar. Section of the rami communicantes in the lumbar region should then abolish sympathetic innervation to the lower limb. Some fibers might still go down the cord and emerge with the sacral nerves but this was of course not possible in those experiments in which the entire lumbar chain was removed. The operations were made under ether anesthesia and the usual aseptic conditions and recovery in each case was without incident. At varying times from 33 to 77 days the dogs were anesthetized and decerebrated, and tests made for the presence or absence of plastic tonus. An autopsy was made on each animal to verify the removal of the sympathetics.

Post-operative observations. The animals were kept all the way from 33 to 77 days after sympathectomy. A careful examination was made in each case before operation and this was repeated several times afterwards. The dogs were all in excellent condition, lively, and in their running and jumping no constant peculiarity in use or position of the operated side could be noted. No difference in tonus could be detected in the leg muscles of the operated side and the various diameters of the leg remained unchanged. On being held up by forepaws each animal walked freely backwards and forwards on its hind legs. In some cases the steps taken by the denervated leg were slightly less and more awkward than those of the control side. Since this did not appear in dogs operated on by the abdominal approach but only in those with the lumbar incisions it was believed to be due to scar tissue which had been formed and could plainly be felt at the crista of the ileum. No difference in resistance to the hand could be found by pressing against the bottom of the feet. When placed on their back and the head held pointing straight in the sagittal plane no constant difference could be detected in the resistance to pressure of the two legs or in the traction necessary for extension. At times one or the other leg seemed to show variations that were significant, but these would fade away or become reversed during the period of examination. In our opinion differences of this kind must be very marked in an otherwise normal dog to have any real significance. The knee jerks never varied as the result of the operations. The results of all our post-operative observations on these animals were entirely negative in so far as the detection of any constant differences between the control and operated side was concerned.

Physiological test for plastic tonus after sympathectomy. So long as an animal has voluntary somatic control of its muscles it is practically impossible to determine the presence of variations in plastic tonus. The phenomenon was discovered by Sherrington in decerebrate animals and it is in such preparations only that the condition can be accurately estimated. To find whether or not the removal of the lumbar sympathetics had any effect on the plastic tonus of the leg muscles of the same side we have

Fig. 1

Fig. 2

Fig. 1. Record of knee jerks. A, from right leg before, and B, after decerebration. The lumbar sympathetics were previously removed from this side. In B the "shortening reaction," characteristic of plastic tonus, is very evident.

Fig. 2. The "shortening reaction" of plastic tonus after the removal of the lumbar sympathetics.

applied the technique suggested by Viets (1920) in his study of the knee jerk and muscle tonus.

After complete recovery from the removal of the sympathetics, the time varying from 33 to 77 days, each unanesthetized dog was placed on its back and tested for knee jerk in each hind leg. By means of a thread fastened to the heel and carried over a series of pulleys a record was made of the movements on a smoked drum. As may be seen in figure 1A, knee jerks recorded in this way show a steep up-stroke representing the con-

traction and an almost equally rapid fall to the base line representing the relaxation. In no case did we secure in either leg the phenomenon of the "shortening reaction" which Sherrington's work indicates as characteristic of plastic tonus. The knee jerks were secured by the light tap of a rod on the patellar tendon.

Following this examination the animals were placed under ether anesthesia and decerebrated. Decerebration was accomplished by inserting a ligature needle through a trephine opening in the parietal region, thrusting it backwards until the tentorium was reached and then directly across the brain stem. The autopsies showed that a transection was made in this way just posterior to the superior colliculus.

Although, as is well known, the dog does not always give the classic picture of decerebrate rigidity so well seen in the cat, certain characteristics of the condition have always been found by us to be well-marked and constant. Immediately after decerebration there was flaccidity and absence of knee jerks on both sides. Within a few minutes the jerks returned and a spastic condition began to appear. We quite agree with the criticism that spasticity might be due to somatic innervation and should not be mistaken for plastic tonus. Our first test was then to determine merely by inspection if the legs would stay put at any given degree of contraction or extension. So well did both the normal and sympathectomized legs do this that two of the animals were used as demonstrations for large medical classes.

Our second test was to see if the character of the knee jerk had altered on the operated side. To do this the knee was held by a rod through the condyle of the femur and the heel connected as before to muscle levers by means of pulleys, and a graphic record taken so that the nature of the relaxations could be determined. Such preparations, if plastic tonus is present, show a marked retardation in the phase of relaxation, and if a series of tendon taps are given, the resulting contractions mount upwards in a stair-step manner. This is of course an expression of Sherrington's "shortening reaction" which we are therefore using as our crucial test for the presence or absence of plastic muscle tonus.

In figure 1A may be seen records of knee jerks from a sympathectomized dog before either anesthesia or decerebration. In figure 1B after decerebration the element of plastic tonus has become very prominent as is evident from the maintenance of the shortened position of the muscles. These records were made from a leg that had had the sympathetics removed 67 days previously. Figure 2 illustrates a still more marked degree of plastic tonus in a leg which had the sympathetics removed 37 days previously. In no case did we fail to secure evidence of plastic tonus.

Autopsies were made on all animals to see if all the rami had been removed. In three experiments there were some small rami found in the

adhesions about the remaining sympathetic trunk. We are certain these had been severed but apparently not avulsed and had subsequently become fixed in the scar tissue. In the others the removal was clean. It is to be remembered that the entire chain was taken out in three of our experiments. The presence of a single branch might possibly throw out three of our records but in nine experiments there is no such objection and we are forced to conclude that in the dog at least the sympathetics are not necessary for an exhibition of plastic tonus.

We have no desire to discuss the entire subject of muscular tonus. It seems to us however that before the theory of the sympathetic origin of plastic tonus through special muscle fibers can be accepted histologists must abundantly confirm the duality of muscle and its innervation and physiologists must secure much better evidence than now exists in favor of the hypothesis. It is at least doubtful if there exists as yet sufficient physiological evidence to justify sympathectomies for the relief of spastic paralyses.

SUMMARY

A series of experiments has been made on dogs to find if the lumbar sympathetics influence plastic tonus in the muscles producing the knee jerk. In six animals all the rami on one side from the second to the sixth lumbar inclusive were severed. In three other animals a few branches of the rami may have escaped. In three experiments the entire lumbar chain on one side was removed. In none of these animals could any muscular disturbance be noticed by observation. After decerebration the "shortening reaction" of Sherrington appeared in all cases on the operated as well as the unoperated side. The conclusion has therefore been drawn that in the dog, plastic tonus does not depend on sympathetic innervation.

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STUDIES ON THE CONDITIONS OF ACTIVITY IN ENDOCRINE GLANDS

XVII. The Nervous Control of Insulin Secretion

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Nervous control of externally secreting glands has long been known and is readily demonstrable. Evidence is not wanting that some of the internally secreting glands also are subject to nervous government, although the necessarily indirect methods of testing have made difficult the securing of conclusive proof. The recent great interest in the rôle of the pancreas in carbohydrate metabolism has raised again the question of a nervous influence on the output of insulin from the islets of Langerhans.

The possible control of the internal secretion of the pancreas through the vagus was suggested several years ago by Eppinger, Falta and Rudinger (1908). Experimental indications of such control were reported by de Corral (1918) in a brief series of investigations on dogs, a slight reduction of blood sugar being obtained on stimulation of the vagus nerve in five out of eight cases. Besides the fact that the anesthetics used (ether and morphine or ether and urethane) are now well known to affect considerably the blood sugar, the periods of observation averaged only a little over an hour, in which time marked changes in blood sugar normal to the preparation, as shown later, invariably occur. Only two controls were reported, and these showed noteworthy variations in blood sugar. The results were therefore far from conclusive, as pointed out by McCormick, Macleod and O'Brien (1923). These observers performed a large number of experiments on etherized dogs and decerebrate cats, and in two cases used rabbits in which the vagus nerve had been exposed in a preliminary operation. The vagus was stimulated in either the cervical or the thoracic or the abdominal region by induced shocks of varying strength and frequency. They obtained only one positive result, however, and their experiments were admitted to be no more convincing than those of de Corral. Both sets of observations merely indicated that the vagus may influence the secretion of insulin.

Two papers by Clark (1924, 1925) have recently appeared showing

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that certain drugs which stimulate the parasympathetic system cause a lowering of blood sugar in the rabbit, and that this effect is not produced in the majority of cases after section of the right vagus. From these findings it was suggested that stimulation of the vagus causes a secretion of insulin. Clark noticed, however, that no altered response to the intravenous injection of glucose occurred after vagotomy.

In contrast with the foregoing, Allen (1922) observed that complete separation of a pancreatic remnant from its original nerve supply in dogs failed to give rise to diabetes, although the possibility of a diminished antidiabetogenic potency because of the lack of a special nervous control was admitted. Banting and Gairns (1924) also concluded, from one pancreatic graft experiment in which the animal showed no diminution of sugar usage, that the secretion of insulin did not necessarily depend on the nerve supply to the islet cells. Destruction of the nerves to the pancreas by section of the duodenal mesentery in frogs was found by Pflüger (1907) to produce glycosuria, but Schwartz and Aron (1925) were not able to confirm this result.

Worthy of particular notice are a number of careful histological studies on the innervation of the pancreas. McCrea (1924) traced branches of the right vagus ("posterior vagal trunk") to the pancreas in man, and pointed out that the supply is similar in the dog, cat and rabbit. Two sets of medullated nerve fibers from the vagus have been shown by de Castro (1923) to be distributed to the pancreas; plexuses of such fibers surround the islets, the actual cells of which are innervated by non-myelinated fibers. Gentes (1902) found that nerve filaments from the peri-insular network proceed to the central part of the islet and there form a very rich intra-insular network. Isolated fibers and small nerve trunks have been shown by Pensa (1904, 1905) to penetrate the islets and follow the vessels between the columns of cells; such fibers become varicose and very tortuous and at their terminations penetrate the columns and lie between the cells. Such a wealth of neural elements, intimately related to the islet cells of the pancreas, is obviously of great significance and demands an explanation in terms of function. Toward the solution of this problem the purpose of this investigation was set.

It was considered possible that previous failures to secure definite results might have been traceable to some of the following complicating factors of which sufficient cognizance had not been taken. The ordinary anesthetics as employed by de Corral and others, for instance, besides elevating the blood sugar above normal also tend to produce wide variations in the glycemic level (Mauriac and Aubertin, 1924; McCormick *et al.*, 1923). These variations are dependent in part, probably, on different degrees of narcosis. It is obvious, nevertheless, that the results obtained would vary similarly. Again, the stimulation of the peripheral

end of a nerve with such a general distribution as that of the vagus might evoke hyperglycogenolysis indirectly unless rigid conditions were instituted. Attempts were made by the earlier observers, indeed, to control this possibility in their experiments by destruction of the hepatic nerve plexus. No consideration was given, however, to the probability of a hyperglycemia resulting from either direct or reflex stimulation of medulliadrenal secretion (Cannon and Britton, 1925) under the experimental procedures involved. Such an event is the more certain because of the very intimate relationship of the abdominal sympathetic and the vagal distribution, that scarcely allows strong electrical stimulation of the latter apart from the former; evidence in support of this is, indeed, furnished hereinafter. In consideration of these hazards, therefore, the rôle of the adrenal mechanism in effecting glycogenolysis has been especially recognized throughout the present investigation.

Methods. The experiments were performed on large and vigorous cats, generally weighing between 3.0 and 4.5 kilograms. Such animals were well able to spare the small quantity of blood used in making the analyses without danger of vitiating the results.

To overcome one of the difficulties experienced by previous workers, the anesthetic, iso-amyl-ethyl-barbituric acid (amytal), was employed. This has been shown by Page (1923) to have little or no influence on blood sugar when used in amounts sufficient to produce surgical anesthesia. Testimony to this fact is also furnished by a number of investigators (Edwards and Page, 1924; Albritton, 1924) and its use in the present study has been fully justified. A dose of 60 mgm. per kilogram body weight administered in a 10 per cent solution by intraperitoneal injection ensured very satisfactory narcosis lasting from ten to thirty hours.

In order to eliminate the hyperglycemia which was found to result from active digestion during an experiment, cats which had fasted from twenty-four to forty-eight hours were used. Although by no means rendering an animal glycogen-free, this procedure, as well as the particular anesthetic used, tended to give much more regular blood-sugar values following the operation.

A normal and practically constant rectal temperature (*circa* 38°) was maintained by means of an electrical heating pad on which the animal rested. Pulse and respiratory rates and blood-pressure readings were taken during the course of the experiments. Corroborating the evidence of Edwards and Page (1924), iso-amyl-ethyl-barbituric acid was found to be particularly favorable to the maintenance of normal blood pressure, readings between 85 and 135 mm. Hg being recorded over periods of ten to thirty hours. Diminution of blood sugar accompanying low blood pressure has therefore been avoided. The ready response of superficial and deep reflexes, which were tested from time to time, was further proof of the general good condition of the animal.

One cubic centimeter of whole blood was used for making the analyses. This amount not only permitted determinations to be made in duplicate, but also constituted a small blood loss (relative to the size of the animal) during an experiment. Samples were taken before and after the operation, and usually hourly thereafter, or more frequently when the blood sugar was believed to be changing rapidly. The blood was drawn from the right femoral artery by means of a glass cannula tied in place just distal to the profunda branch. The cannula held slightly more than 1 cc., and control by an arterial clamp allowed the desired amount to be withdrawn. A very small quantity of powdered potassium oxalate was used as an anticoagulant. Blood sugar was determined by the method of Folin and Wu (1920). Precipitation and filtration were made after the withdrawal of each blood sample; the protein-free filtrates were kept until the end of an experiment and reduction carried out in all at the same time, comparison then being made with the same standard.

As already pointed out, morphological as well as physiological evidence indicates that the internal secretion of the pancreas is mediated through the right vagus. This nerve has been used in the present work, the main trunk on the posterior aspect of the cardia being chosen as the most favorable site for stimulation. By careful dissection through a median incision in the upper abdomen the nerve was freed for 1.5 to 2 cm. and tied and cut above; the branches to the stomach and upper intestine were also cut; then rubber shielded electrodes were applied to the main peripheral trunk proceeding to the celiac plexus and celiac and mesenteric arteries. The use of this method has circumvented the fall of blood pressure due to stimulating the vagus in the neck, or the interference with respiration due to stimulating the nerve in the thoracic region.

It is to be emphasized that in dissecting the vagus as described above care was exercised to avoid injuring the nerve by tension, and also that the perineural sheath was left as far as possible intact. The latter condition necessitated the use of a fairly strong electrical stimulus, from 300 to 700 Z units being generally used. The induction shocks were varied in frequency from 5 to 10 per second. Usually the stronger stimuli and higher frequencies were found more effective. Different periods of stimulation as detailed later were employed.

In a few of the earlier experiments both adrenals were eliminated; the adrenal vein was first clamped and the surrounding peritoneum then dissected to allow free exposure of the pedicle for tying before the excision. The nerves proceeding to the liver along the hepatic artery were in these cases also severed, although thereby some of the fibers distributed to the head of the pancreas were interrupted. Nevertheless the greater amount of islet tissue known to be concentrated in the caudal end of the pancreas still had its innervation intact. Preparation of the vagus for

stimulation was carried out as already described, and in all instances the abdomen was then carefully closed around the electrodal leads to the nerve. Such operative procedures usually took from 30 to 45 minutes, after which the animal was kept free from all disturbance except that of stimulating the vagus and that of withdrawing the blood sample.

Results. The results of these experiments are given in table 1, group A. It is observed that from the fairly high blood-sugar values obtaining for some time after the induction of anesthesia, well-marked diminution occurred in each case on stimulating the vagus. In three experiments

TABLE 1

The effect of vagus stimulation on blood sugar

Group A: Both adrenals inactive. *Group B:* Both adrenals active. Hepatic nerves cut in all cases.

GROUP AND DATE		EXPERIMENT NUMBER	WEIGHT OF CAT	TIME STIMULATION STARTED AFTER ANESTHETIC		DURATION OF STIMULATION		BLOOD SUGAR		
								Before stimulation	After stimulation	Change
			kgm.	*hours*	*minutes*	*hours*	*minutes*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*
A	12–15–24	1	3.0	2	55	1	06	193	142	−51
	12–16–24	2	1.9	1	40	0	45	199	172	−27
	12–19–24	5	3.4	2	55	1	15	232	164	−68
B	12–18–24	4	2.5	2	10	1	05	157	179	+22
	1– 8–25	6	4.0	2	55	0	30	218	203	−15
	1– 8–25	6	4.0	3	50	1	00	194	174	−20
	1– 9–25	7	4.4	2	45	1	00	156	170	+14
	1– 9–25	7	4.4	5	15	1	30	156	144	−12
	1–14–25	9	2.8	4	15	2	00	154	145	−9

Average decrease of blood sugar on vagus stimulation with both adrenals inactive (group A), 47 mgm. per hour.

Average decrease of blood sugar on vagus stimulation with both adrenals active (group B), 3 mgm. per hour.

the average fall was 47 mgm. per hour. The controls, however, in which precisely the same operative detail was carried out, indicated that these results could not be interpreted as wholly due to vagus effects, since a gradual decline in blood sugar frequently took place following bilateral adrenalectomy when no stimulus was applied. No conclusions were therefore drawn from these results, although they were considered as being suggestive.

In another group of experiments (table 1, B) the hepatic nerves were cut but both adrenals were left intact. In a few cases small decrements in blood sugar were observed on vagus stimulation. It was obvious from these cases, however, and especially from two in which slight increases

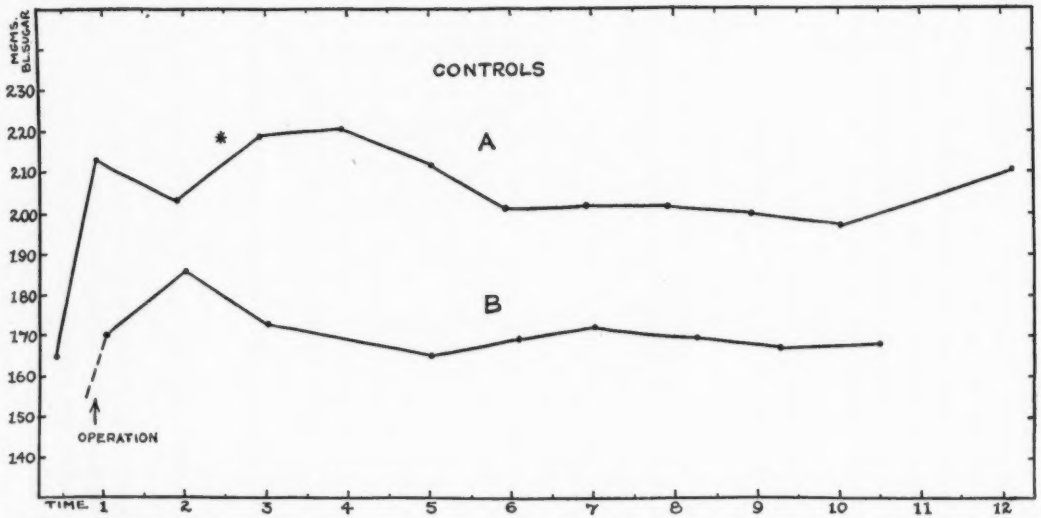

Fig. 1, *A* and *B*. Curves representing the course of blood sugar in two control experiments. Amytal anesthesia (as in all figures) at zero time. *Hepatic nerves cut* in each and right vagus nerve prepared as for stimulation; in *A*, left adrenal also inactivated. Following the initial disturbance due to operative trauma, fairly constant blood-sugar values occur over periods of several hours. The light anesthesia maintained from the outset in *A* resulted in a slight irregularity of the sugar level with a tendency to increase until controlled by a second dose of amytal (at *).

occurred, that under the conditions no more satisfactory results could be expected than those reported by previous workers. The glycemic level nevertheless remained practically unchanged in one control animal for several hours after the usual initial rise due to laparotomy, as shown in figure 1, *B*.

The impression thus became more strongly established that varying adrenal activity was probably responsible for the inconstancy of the re-

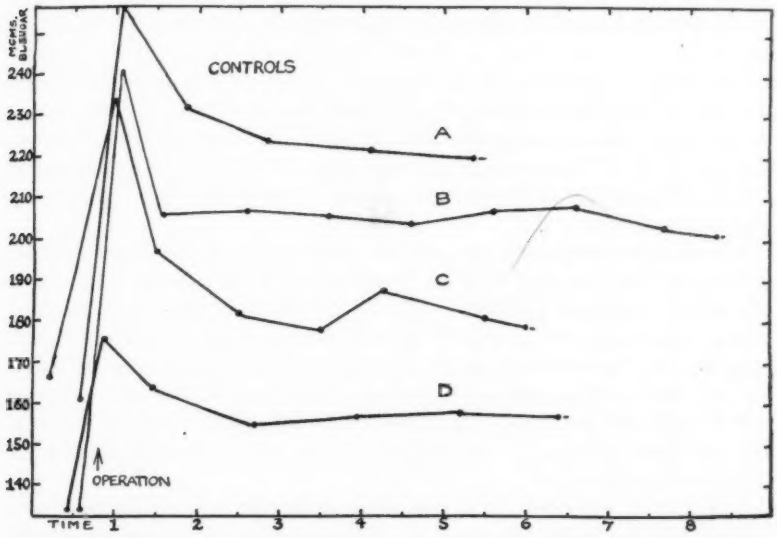

Fig. 2, *A*, *B*, *C*, and *D*. Four control experiments showing the course of blood sugar under amytal. *Hepatic nerves not cut except in D.* In all cases the left adrenal was completely tied off, and right vagus nerve prepared as for stimulation. The operative procedures invariably produce a marked increase in blood sugar and the eventual level assumed, and maintained for several hours, is correlated with the peak of increase.

sults obtained, and with this in mind a third series of experiments was undertaken.

It was noted that the main trunk of the right vagus in its course through the abdomen assumed in the cat a sinistro-lateral position, with fibers going chiefly to the left celiac and renal plexuses. The idea was therefore entertained that either direct or indirect adrenal activity on vagus excitation might be aroused, particularly in the gland on the left side. In table 2, group C, is given an epitome of the results of eight experiments in which the hepatic nerves were cut and the left adrenal inactivated by means of simple block ligature—a method which effected an economy in

time of operation. The major and minor left splanchnic nerves were also severed and the hepatic nerves cut in the pedicle in experiment 37, and the right adrenal was inactivated in experiment 17. These experiments represent eleven cases of vagus stimulation.

In order to ensure an even blood-sugar level before application of the stimulus, a period of three hours or more was allowed to elapse after the administration of the anesthetic. The necessity for this precaution will be appreciated on glancing at the control sugar curves given in figures 1, *A* and *B*, and 2, *A*, *B*, *C* and *D*, to be treated more fully later. The stimulation usually lasted from one to two hours, and occasionally three hours. Of important significance is the fact that in group C vagus excitation was followed in every instance by a reduction in the percentage of blood sugar. In one case when stimulation was applied in three periods at intervals the low level of 81 mgm. per cent was reached. A general agreement in the rate of decrease of blood sugar will also be noticed, the average being 18 mgm. per hour.

The results tabulated in group D, in which the same conditions as those in group C were present with the exception that the hepatic nerves were left intact, represent findings which are substantially similar to the foregoing. During every period of vagus stimulation the blood sugar consistently fell, the rate of diminution being somewhat greater than in group C. In the six experiments which were carried out the average decrease was 27 mgm. per hour. It will be observed that a blood sugar of 87 mgm. per cent was produced in one instance—again a low value under the experimental conditions.

Evidence in corroboration of the latter statement is furnished by a number of control experiments, examples of the results of which are graphically reproduced in figures 1, *A* and *B*, and 2, *A*, *B*, *C* and *D*. The course of the blood sugar is shown for periods ranging from six to twelve hours in six experiments. In point of operative procedure they are representative of those in which the vagus nerve was stimulated (tables 1 and 2). In some of the shorter controls, in fact, vagus stimulation was subsequently carried out. Fairly wide fluctuations in the curves are seen to occur during the period of operation (laparotomy, etc.); these can be attributable only to the operative disturbance. At the end of the second or third hour the blood sugar usually assumes a level from which only negligible deviations occur, and thus continues, as shown, for considerable periods. Further interference with the preparation or the eventual setting in of disintegrating conditions is indeed alone responsible for any variation, provided a sufficient amount of the anesthetic has been administered at the start of the experiment. In one case (fig. 1, *A*) when the animal was purposely given an initial light dose of iso-amyl-ethyl-barbituric acid, general body movements occurred now and again, and at this time the curve showed a

slight upward tendency. The condition was readily controlled, however, by a second small dose of the narcotic.

The different blood-sugar levels assumed, as well as the particular height of increase which occurred at the time of operation, were probably dependent on the varying amounts of glycogen held in reserve by the animals,

TABLE 2

The effect of vagus stimulation on blood sugar

Group C: Hepatic nerves cut. *Group D:* Hepatic nerves intact. One adrenal inactive in all cases.

GROUP AND DATE		EXPERIMENT NUMBER	WEIGHT OF CAT	TIME STIMULATION STARTED AFTER ANESTHETIC		DURATION OF STIMULATION		BLOOD SUGAR		
								Before stimulation	After stimulation	Change
			kgm.	*hours*	*minutes*	*hours*	*minutes*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*
C	1-20-25	11	4.2	7	55	1	05	144	127	−17
	1-21-25	12	4.0	3	00	1	30	194	151	−43
	1-23-25	13	4.4	3	50	1	00	203	185	−18
	1-23-25	13	4.4	7	35	2	00	163	128	−35
	1-23-25	13	4.4	12	20	0	55	92	81	−11
	1-28-25	14	3.6	7	05	1	00	233	212	−21
	2- 3-25	16	3.8	4	30	3	00	202	164	−38
	2- 5-25	17	4.1	4	15	1	30	175	157	−18
	2-10-25	19	3.7	4	30	2	25	178	142	−36
	2-12-25	20	3.1	3	50	2	10	221	179	−42
	4-14-25	37	4.2	8	10	1	00	136	110	−26
D	2-16-25	21	4.2	3	25	2	15	228	170	−58
	2-16-25	21	4.2	7	45	0	25	170	134	−36
	2-26-25	24	4.4	5	15	3	15	202	140	−62
	3-10-25	29	4.6	6	00	2	00	179	133	−46
	3-25-25	32	3.1	4	25	1	40	130	87	−43
	4- 1-25	33	2.6	9	15	1	00	248	205	−43

Experiments 17 and 32, right adrenal inactive and left splanchnic nerves cut; in all other cases, left adrenal inactive. The left splanchnic nerves were cut in experiment 17.

Average decrease of blood sugar on vagus stimulation with one adrenal inactive. Hepatic nerves cut (group C), 18 mgm. per hour. Hepatic nerves intact (group D), 27 mgm. per hour.

plus the individual response to operative trauma, as by sympathetic and adrenal discharge. In this connection it is also noted that a definite correlation exists between the peak of blood sugar reached in the preliminary rise and the height of the eventual plateau. Explanation is therefore now at hand for some of the cases given in tables 1 and 2 in which the glycemia was slightly high at the time of stimulation; it will be appreciated that the normal for the conditions of the preparations was as far as possible ascertained.

Several figures illustrating the course of the blood-sugar curve attending vagus stimulation are reproduced (see figs. 3 to 9). These are taken as typical in the series. They are in striking contrast to the controls, marked depressions from the normal level occurring in all instances. At the higher glycemic levels, as revealed by a comparison of figures 3 and 4

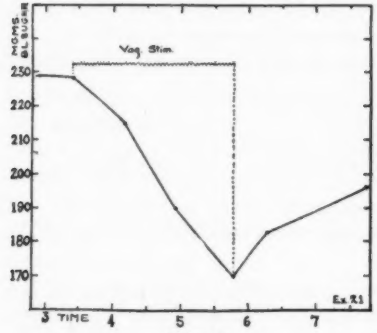

Fig. 3

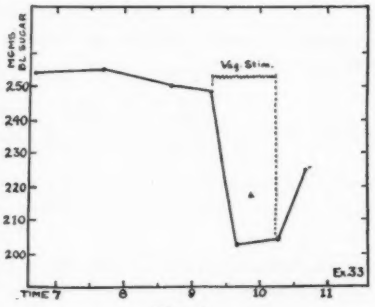

Fig. 4

Figs. 3 and 4. Effect of vagus stimulation on blood sugar. Left adrenal tied in each case. Note more rapid blood-sugar fall from higher level in figure 4. Compare with figures 5 and 6, and see also text.

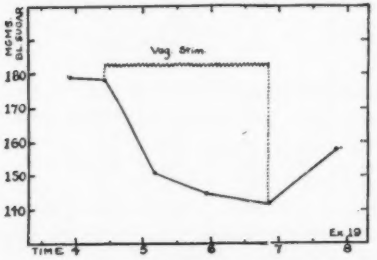

Fig. 5

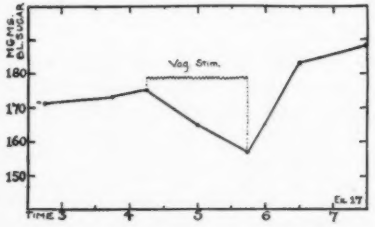

Fig. 6

Fig. 5. Effect of vagus stimulation on blood sugar. Left adrenal tied and hepatic nerves cut. The rate of decline diminishes at the lower blood-sugar levels. Cf. figures 3 and 4.

Fig. 6. Effect of vagus stimulation on blood sugar. Hepatic nerves cut, left adrenal tied, and left splanchnic nerves cut. Cf. figures 3 and 4.

with figures 5 and 6, vagus stimulation is much more effective. Hence the decline from 228 mgm. and from 248 mgm. (figs. 3 and 4) is much more sharply defined than that from 178 mgm. and from 175 mgm. (figs. 5 and 6). In these respects the similarity to insulin action is particularly noticeable (see pp. 301–2).

Throughout the period of stimulation the blood pressure, respiration

and pulse readings showed only small variations. In test experiments, when a very strong stimulus was applied, an immediate rise in blood pressure occurred, similar to that taking place on splanchnic stimulation and apparently indicating a spread of current from the vagus to the sympathetic system. Such a strong stimulation was avoided in the regular course of the work.

In a few experiments in which blood samples were taken more frequently a brief latent period with slight or no decline in the blood-sugar level following stimulation was sometimes observed. Occasionally a small decline persisted beyond the point at which the stimulus was withdrawn. The usual observation, however, was that indicated by the majority of the graphs—an immediate depression accompanying vagus stimulation and a rather sharp rebound from the consequent low sugar level after the stimulus was stopped (see fig. 7). In some cases this release recovery approximated to or rose beyond the original resting level.

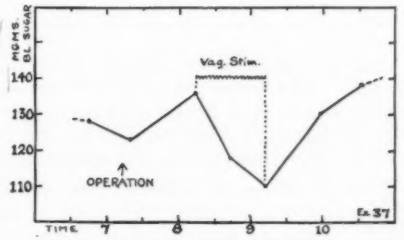

Fig. 7. Effect of vagus stimulation on blood sugar when the curve was rising after abdominal operation. Left adrenal tied, left splanchnic nerves cut, hepatic nerves cut. Note rapid changes of glycemic concentration at the start of stimulation and after stimulation was stopped.

The effect on blood sugar of section of the right vagus at the cardia has been observed in three experiments. In each case the nerve was carefully exposed and looped with two threads; these were then drawn through a conical paraffined guard leading from the exterior to the nerve *in situ*. The abdominal wall was closely sutured around the guard, and thus the cutting of the nerve without other disturbance of the animal was a simple matter. Figures 8 and 9 illustrate the trend of the blood sugar following vagotomy. In one instance (fig. 8) a fairly marked rise occurred, while in the other (fig. 9) a moderate increment is perceptible, before the conditions were altered by vagus stimulation. In the third experiment an irregularity of the curve with a tendency to rise was produced on vagal section. In contrast with these findings is the indication given in figure 8 that ligature of the vagus (equivalent to temporary stimulation) resulted in a transient depression of blood sugar.

Comparison with insulin action. The effect of vagus stimulation has been compared with that of insulin in a few experiments on amytalised animals. To provide similar conditions the left adrenal was tied and the vagus prepared as for stimulation in one instance. Following the intravenous injection of insulin in a dose of 10 units per kilogram weight the

blood sugar fell from 209 to 61 mgm. per 100 cc. in three and a half hours. The decline was rapid at first and became considerably slower during the last two hours—results which are remarkably similar to those occurring

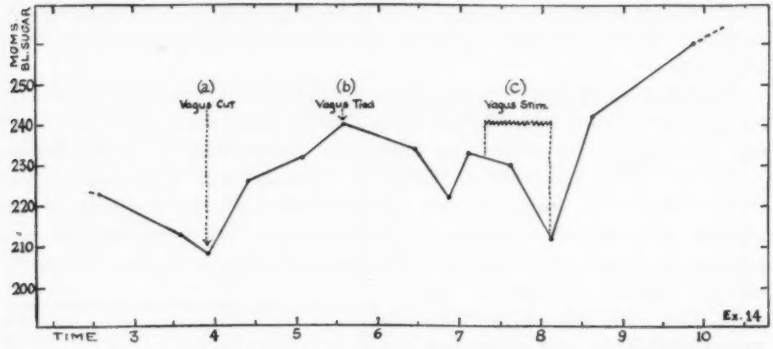

Fig. 8. Effects on blood sugar of *a*, cutting; *b*, tying; and *c*, stimulating the right vagus. Left adrenal tied and hepatic nerves cut. A well-marked rise follows section of the nerve (withdrawal of nervous impulses); the blood sugar falls on simple ligation (equivalent to temporary stimulation), and a further fall occurs on vagus stimulation. The rise after stopping stimulation is very sharply defined in this case.

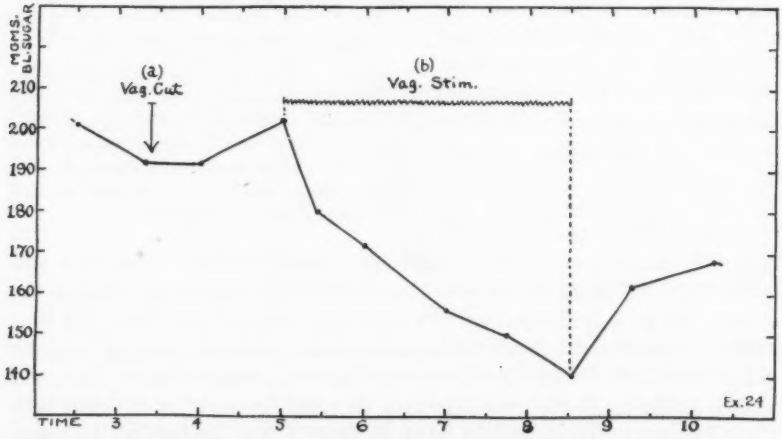

Fig. 9. Effects on blood sugar of *a*, cutting, and *b*, stimulating the vagus. Left adrenal inactivated. The curve begins to rise soon after vagus section, but stimulation is accompanied by a persistent lowering of the blood sugar and is followed by a rise.

on vagus stimulation, as shown in the accompanying figures. The average rate of decline on insulin administration was 42 mgm. per hour. This rate of blood-sugar fall was approximated and even exceeded in a few cases when the vagus was stimulated.

Controls with pancreas inactive. Further controls were carried out for the purpose of determining the effect of vagus stimulation on blood sugar when the pancreatic vessels and accompanying nerves were tied. An abstract from one experiment is given below.

Experiment 27a. March 5, 1925.

Cat, male, 2.6 kilos. Amytal anesthesia.

Right vagus (on cardia) cut and tied and shielded electrodes applied to peripheral end; hepatic nerves cut, left adrenal tied, and superior and inferior pancreaticoduodenal arteries and veins tied; abdomen closed.

TIME	CONDITIONS	BLOOD SUGAR
		mgm. per 100 cc.
6:45 p.m.	2¼ hours after operation and before stimulation	134
7:45 p.m.	After one hour's stimulation of right vagus	134
9:20 p.m.	Stimulation withdrawn	132
10:20 p.m.	No stimulation	131

Autopsy showed that all the pancreatic vessels were tied off. The pancreatic tissue was venous in appearance.

In another control experiment similar to the above in which all the pancreatic vessels were tied but the hepatic nerves were not severed, the blood sugar was 261 mgm. per 100 cc. before stimulation and 262 mgm. at the end of 45 minutes' stimulation of the right vagus.

It was therefore apparent that the diminution of blood sugar on vagus stimulation depended on the physiological integrity of the pancreas.

The possibility of the depletion of hepatic glycogen. The observation that a sharp rise in blood sugar usually occurred after stopping vagus stimulation contra-indicated this conception. As an additional means of determining whether the glycogen reserves were seriously affected during the course of experiments, which frequently extended over several hours, many of the well-known measures which have been shown to produce hyperglycemia were tested just before destroying the animal with ether. In the large number of tests made it was found that afferent nerve stimulation, operative procedures or trauma, hemorrhage, the injection of adrenalin, and asphyxia produced rapid and often marked increases in the blood-sugar level.

Pancreatic circulation on stimulating the vagus. The possibility of differences in the rate of blood flow through the pancreas occurring on vagus stimulation has been considered. It may be noted that several years ago Anrep (1916), using a different strength of stimulus, was unable to detect vaso-dilator fibers in the vagus. In the present investigation two experiments were carried out in which the cannulation of the pancreatic veins allowed an index of blood flow through the gland to be obtained. The ordinary drop-count method was used, and determinations

were made at intervals and during several periods of vagus stimulation. Various intensities and frequencies of stimulation, as used throughout the course of the work, were applied. Other experimental procedures and results are cited below in brief.

Experiment 25a. March 3, 1925.

Cat, male, 3.3 kilos. Amytal anesthesia.

Right vagus cut and tied and electrodes applied to peripheral end, hepatic nerves cut, left adrenal ligated, inferior pancreatico-duodenal vein cannulated.

Count of drops from cannula:

	Drops per 30 seconds
Resting condition	8.0–7.5–7.5
Stimulation of vagus	7.5–7.5–7.5
No stimulation	7.5–7.0–7.0–6.5
Stimulation	7.0–7.0–7.0–6.5
No stimulation	7.0–6.5
Stimulation	7.0–6.5–6.5
No stimulation	6.5–6.5–7.0–6.5
Stimulation	6.5–7.0–6.5

After five periods of vagus stimulation extending over twenty minutes the count of drops varied between 6 and 7 per 30 seconds.

In experiment 26a the superior pancreatico-duodenal vein was cannulated and other operative measures carried out as in the foregoing. The blood flow varied between 5.0 and 5.5 drops per 30-second period after the first two "resting" counts, and no change occurred during vagus stimulation.

Effects of sympathetic stimulation. It is well known that other glands, e.g., the salivary, are supplied by two sets of nerve fibers, each of which has an effect on the mechanism of secretion. In order to determine the possible presence of either a similar or an opposing influence of the sympathetic system on the blood sugar, in comparison with that of the parasympathetic (vagus) action, the splanchnic nerves (major and minor) and the sympathetic nerves along the inferior pancreatico-duodenal artery have been stimulated under various conditions in eight experiments. In all cases the left splanchnic nerves were used and the left adrenal was inactivated. The results as a whole show that although a marked hyperglycemia occurs on splanchnic stimulation when the hepatic nerves are intact, practically no change in blood sugar takes place if the hepatic nerves are cut. Similarly the stimulation of the nerves along the inferior pancreatico-duodenal artery has been virtually without effect on the blood-sugar level, a slight irregularity of four or five points from the mean being the usual observation.

Discussion. Previous work on the nervous control of insulin secretion has yielded no definite results, probably because of insufficient consideration of the hyperglycemic influence of certain anesthetics and operative procedures incidentally, and of the adrenal mechanism in particular. A

great deal of evidence has been brought forward (Mauriac and Aubertin, 1924; Griffith, 1923; Babkin, 1925) regarding the influence of these factors, and in their presence enormous doses of insulin—from 25 to 35 units per kilo weight—have been shown to be necessary to lower the blood sugar (Edwards and Page, 1924; Hepburn *et al.*, 1924). Cannon, McIver and Bliss (1924) have also demonstrated that there is an increased adrenal discharge in response to nervous impulses under insulin dosage, and that this mechanism is protective against a dangerous hypoglycemia. Using different methods such a stimulation of adrenin secretion by insulin has recently been corroborated by several other workers (Houssay *et al.*, 1924a, 1924b; Abe, 1924). Moreover, evidence for a secondary discharge of adrenin has been adduced by Gutowski (1925), occurring after such stimulation of the peripheral end of the vagus nerve as causes cardiac inhibition and fall of blood pressure.

By taking account of these considerations in the foregoing work it has been found possible to offset an opposing hyperglycemia and indeed to obtain practically unchanging blood-sugar values extending over many hours. From such values consistent depressions were effected in two groups of experiments on stimulation of the right vagus nerve. Results are presented in a number of tables and figures. The blood-sugar curves are found to be similar to those obtained by Banting and Gairns (1924) when they stimulated the islet cells of the pancreas to secrete insulin by the application of heat, and also similar to those obtained by Edwards and Page (1924) and the present writer with insulinised animals under amytal anesthesia. Correspondence to the particular action of insulin (also noted by Allan, 1924), is further observed in the more pronounced effect of vagus stimulation at the higher glycemic levels.

Although in a number of instances relatively low blood-sugar findings occurred on vagus stimulation, in only a few cases were low absolute values induced. In this connection the similarity to insulin is again evident, if, as many workers have indicated (Huxley and Fulton, 1924; Olmsted, 1924; Macleod, 1924), the action of the latter depends on the metabolic rate of the animal. Thus under amytal narcosis, the metabolism being noticeably depressed, as also pointed out by other investigators (Edwards and Page, 1924), the diminished effectiveness of insulin as well as the rather limited effect of vagus stimulation in these experiments may be understood. In the light of recent evidence showing that the tissues of anesthetized animals contain greatly reduced amounts of insulin (Best, Smith and Scott, 1924), the restricted vagus action on blood sugar is further explained.

That the lowering of the blood sugar may possibly occur apart from pancreatic secretion should be considered. The presence of a hypoglycemia-producing hormone in the intestinal mucosa and other tissues has

been suggested, for instance, by the work of some observers (Best *et al.*, 1924; Lombroso, 1924; Ivy and Fisher, 1924). Only very small amounts of "an insulin-like material" and that of limited potency have been obtained, however, and the possibility of the occurrence of artefacts or the lowering of blood pressure and hence of blood sugar (McCormick *et al.*, 1923) by such "material" has not been excluded. Again, Snyder, Wells and Culley (1923) have shown that the vagus may inhibit hepatic glycogenolysis and thus produce hypoglycemia. A positive result, it may be noted, was forthcoming in only one of their experiments. Such remote considerations have been met in the foregoing report by the fact that under the terms of the experiments negative results occurred on vagus stimulation when the pancreatic vessels and nerves were ligated, in contrast to consistent blood-sugar depressions in others in which the connections of the pancreas were intact.

In accord with the present findings are those of Lewis and Magenta (1924) and of Houssay and Lewis (1923), showing that vagotomised in contrast with splanchnicotomised animals are less sensitive to the action of insulin, and manifest a greater hyperglycemia under morphine administration. An interesting report by Brugsch, Dresel and Lewy (1921) also points out that hypoglycemia may result from stimulation of one part of the vagus nucleus in the medulla. That a centre controls the nerves to the pancreas is postulated by these writers. The so-called condition of "hypervagotonia" described by others (Santenoise and Tinel, 1923), in which a high glucose tolerance is invariably found, is again indicative of vagus control of blood sugar.

The possibility of defective utilization of carbohydrates following denervation of the pancreas has been considered. Several experiments already carried out indicate that such a defect may occur. A report of these experiments, together with others now in progress, will be presented later.

SUMMARY

Using iso-amyl-ethyl-barbituric acid as an anesthetic, and either inactivating the left adrenal or severing the left splanchnic nerves, the blood sugar in the cat may be maintained at a constant level for many hours. The stimulation of the right vagus on the cardia in such a preparation upsets this equilibrium and produces a lowering of the blood sugar which persists during the period of stimulation. Blood-sugar curves representing these results are similar to those obtained under insulin administration. On stopping the stimulus the original or even a higher glycemic level may be reached.

The effects are produced when the hepatic nerves are either cut or left intact.

The possibility of exhaustion of glycogen reserves has been excluded.

The circulation through the pancreas is unaffected during vagus stimulation.

If the vessels and nerves of the pancreas are tied and the vagus then stimulated no lowering of the blood sugar occurs.

No reduction of blood sugar occurs on stimulation of the sympathetic nerves to the pancreas under various conditions.

A great deal of evidence emphasizing the rich innervation of the islets of Langerhans is reviewed. The present results show that the internal secretion of the pancreas is affected by nervous influences, and that control of the secretion of insulin occurs through the right vagus nerve.

It is with much pleasure that the helpfulness and inspiration of Prof. W. B. Cannon, at whose suggestion this work was undertaken, are acknowledged.

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SPECTROPHOTOMETRIC ANALYSIS OF COMMERCIAL INSULIN

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Representative samples of commercial insulin were analyzed spectrophotometrically[1] with the original purpose of determining whether any characteristic absorption bands existed in the visible spectrum. It appears that there are no absorption bands in the visible spectrum, but the general nature of the curves showing the transmission plotted as a function of the wave lengths may be of practical significance in the determination of the content of insulin. This will hold true only if the mode of manufacture is kept constant. Readings obtained on U-20 insulin from various manufacturers are shown in figure 1, where it is seen that the curves are all similar but not superimposed, probably on account of the different methods of manufacture.

We have made observations on insulin prepared by the Connaught laboratories, Eli Lilly & Company, B. F. Stearns & Company, E. R. Squibb & Sons, the Allen Hanburys and British Drug Houses, and, according to the method of Dodds and Dickens, by the Mayo Clinic laboratories. The results of our observation lead us to believe that the spectrophotometer may be used to determine the strength of a given sample of insulin.

Commercial insulin, as supplied by the manufacturers today, contains substances other than insulin itself; among them is a yellow pigment. The amount of this pigment may be directly proportional to the strength of the insulin. The so-called U-10 insulin is almost water-clear to the eye; the U-80 insulin is quite yellow; the U-20 and U-40 strengths seem yellow in proportion to their insulin content. When insulin is assayed according to our method it is practically certain that it is a yellow substance other than insulin that is being measured. However, if the color is directly proportional to the strength of the insulin, this makes no difference. If the manufacturers, whose insulin we have analyzed by means of the spectrophotometer, continue to make their insulin according to their present

[1] The spectrophotometer used was the "color analyzer" of Keuffel & Esser. This instrument is described by Wallace R. Brode, Amer. Chem. Journ., 1924, xlvi, 581.

method, they may be able to estimate the strength of that insulin by our method. Should they change their present method of manufacture, new curves can be plotted and the spectrophotometric assay may still be valid. It will be noted from figure 1 that curves for the products of two firms are so similar as to be practically identical when allowance has been made for laboratory error. Apparently the products by two different methods show the same ratio of insulin to pigment.

Every sample of insulin in our experiment was compared with distilled water. Water filled one cell and insulin the other. The 10-cm. cell was used in every case. Both cells were kept free from bubbles and every

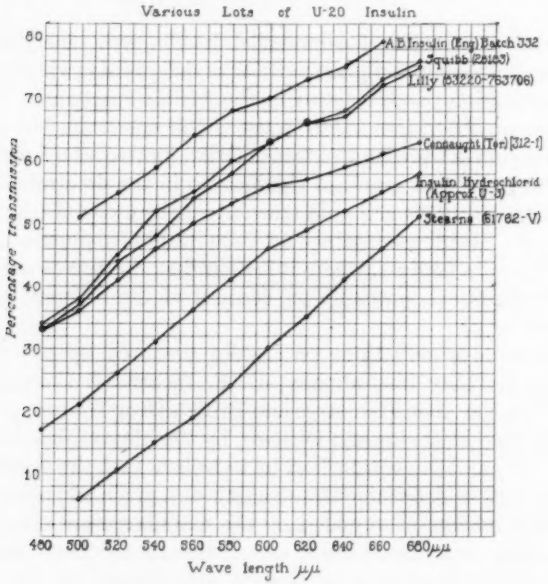

Fig. 1. Spectrophotometric determinations of U-20 from various manufacturers.

effort was made to render them absolutely free from sediment of any sort. We found that the slightest amount of sediment changed the results and that the most reliable way to keep the cells free from sediment was to allow each sample of insulin to stand for several hours in the cell before any readings were made. When the two cells were in place in the spectrophotometer, readings were made at intervals of 20 millimicrons from a wave length of 480 millimicrons to a wave length of 680 millimicrons. Readings below and above these points are difficult to make. Three sets of readings were made on each sample, and the average reading taken as final. An interval of a few minutes was allowed to elapse between the

readings because it was found that the eye fatigue, which resulted if too many readings were attempted without a rest, vitiated the results.

In order to establish so-called standard curves, a sample of U-80 insulin was diluted with N/100 hydrochloric acid to U-40, U-20, U-10 and U-5. The hydrogen-ion concentration was kept constant. Complete readings were made on each. The results, for example, of a representative commercial lot of insulin with the various dilutions are shown in figure 2. When these results are charted it is easy to construct another chart (fig. 3) by

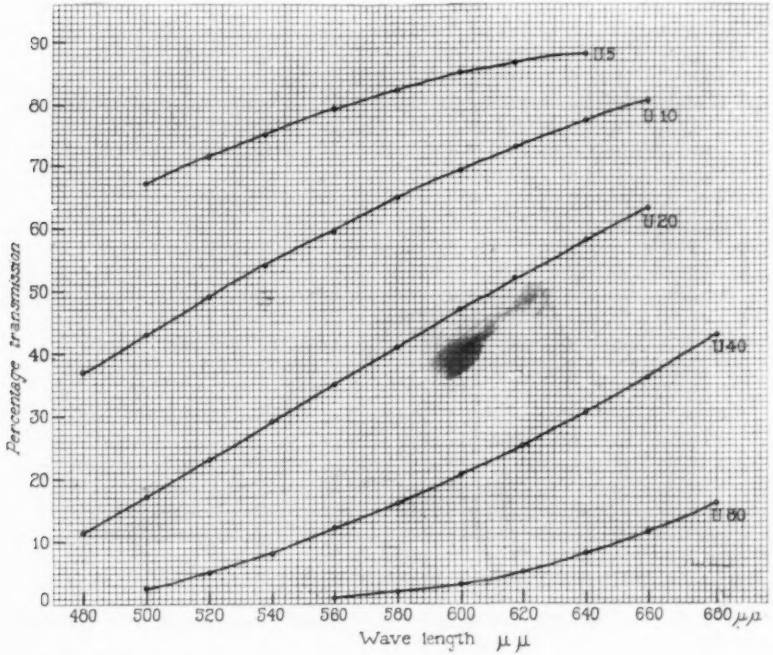

Fig. 2. Spectrophotometric determinations of insulin, with hydrogen-ion concentration constant.

means of which it is possible to read a sample of insulin and to compare it immediately with this chart to determine its strength. When curves are established, readings at intervals of 20 millimicrons need not be made on new samples of insulin. Four readings only are necessary, namely, at wave lengths of 520, 560, 600 and 640 millimicrons. With these four readings it seems possible to determine the strength of an unknown solution of insulin if this insulin has been made by the identical process of manufacture as the insulin which, on analysis, made possible the construction of

the original curves. For example, suppose that a sample of insulin of unknown strength transmits 23 per cent of light at a wave length of 520 millimicrons, 35 per cent of light at a wave length of 560 millimicrons, 46 per cent of light at a wave length of 600 millimicrons, and 57 per cent of light at a wave length of 640 millimicrons. These readings can be applied directly to the chart (fig. 3) where the strength of insulin is plotted against the percentage of transmission, and the number of units of insulin can be read off directly. In this particular case the strength of insulin would be that of U-20.

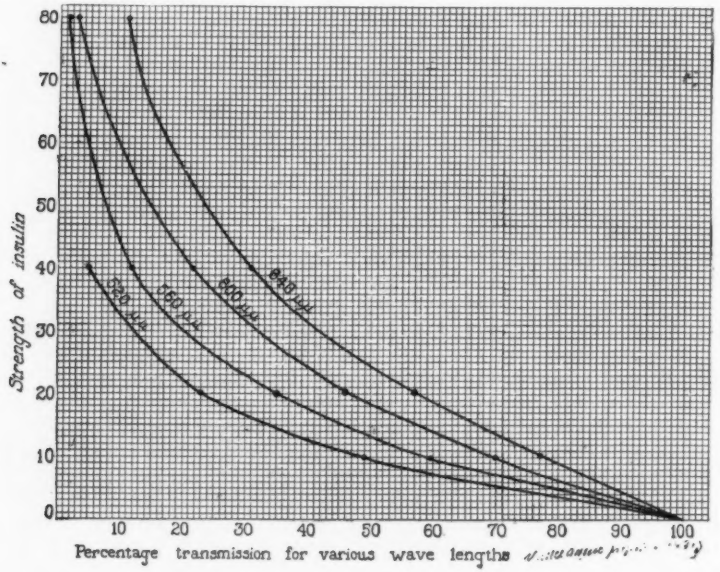

Fig. 3. Curves showing variations of percentage transmission with strength of insulin.

We do not presume to say that this method of analysis is also a means of identification. The existence of insulin in a solution cannot be confirmed or denied by examining that solution by the method here described. What we suggest is merely that the number of units in each cubic centimeter of commercial insulin may be determined quickly and accurately by the method described.

One-tenth of 1 per cent of tricresol was used as a preservative in the insulin we had under consideration. We found tricresol in this strength to give the same absorption curve as distilled water; therefore, its presence did not vitiate the results.

SUMMARY

Commercial preparations of insulin and insulin prepared in our own laboratory show no absorption bands in the visible spectrum.

The general nature of the curves showing the transmission plotted as a function of the wave length may be of practical significance in the determination of the strength of a given sample of insulin. This method may be dependent on the possibility that the amount of yellow pigment in commerical insulin is proportional to the insulin itself.

SOME PHENOMENA OF DECEREBRATE RIGIDITY

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In a series of experiments which we have been conducting upon changes in heart rate, we had occasion to perform complete decerebration upon a number of cats. In these animals, the skull was opened and the cerebral hemispheres removed, but the thalami were left intact. The decerebrate rigidity which ensued was, of course, a constant phenomenon. After subsequent excision of the stellate ganglia by the method devised by Pike and described by Tulgan (1923) which not only does away with injury to the muscles of the shoulders, but obviates the opening of the thorax, it was observed a number of times that the rigidity of the fore limbs was very much diminished, while the rigidity of the hind limbs persisted unchanged. This falls into line with the work of John Hunter upon the sympathetic innervation of skeletal muscle, in which he showed that the condition of spastic rigidity of a limb was very appreciably lessened by section of the sympathetic nerve supply to it.

Another occurrence which we have never seen mentioned in the decerebrate animal is associated with the production of anemia of the medulla and mid-brain by occlusion of the head arteries in the manner described by Stewart (1906). As this anemia progresses, the decerebrate rigidity disappears, its disappearance being first observed in the fore limbs and gradually spreading to the hind limbs. This is what one would predict since it is well known that section of the brain-stem at the level of the inferior colliculi in the cat abolishes decerebrate rigidity, and in this case, anemia rather than trauma has eliminated the mid-brain. What has never to our knowledge been observed before, is that, on restoration of the circulation to the head (artificial respiration being maintained), with the return of the various functional activities of the medulla, there is a *return of decerebrate rigidity*. That is, the brain stem must be regaining its functional activity to at least as high a level as the inferior colliculi.

We have also found that this condition of loss and return of decerebrate rigidity bears a definite time relation to the loss and reappearance of the corneal reflex following occlusion and release of the head arteries. The rigidity of the fore limbs begins to disappear before the corneal reflex is lost, and returns before the reappearance of the corneal. Inasmuch as the

mechanism of the corneal reflex lies chiefly in the cells of the superior colliculi, which contain the terminal nuclei of the external optic tracts, while the establishment and maintenance of decerebrate rigidity is concerned more with the integrity of the inferior colliculi, the time relationships in the disappearance and reappearance of the two reflexes illustrate forcibly the progressive onset and recession of the wave of the phenomena attending anemia above the level of the medulla. The following protocol illustrates this.

June 26, 1925

2:00. Ether, tracheotomy
2:05. Head arteries prepared for occlusion
2:07. Cannula in left carotid; blood pressure recorded
2:15. Decerebration
2:22. Decerebrate rigidity appears
2:28. Corneal reflex present
2:30. Head arteries occluded
2:31½. Decerebrate rigidity of fore limbs has disappeared
2:32. Head arteries released
2:33. Rigidity of fore limb reappears
2:39. Corneal reflex reappears
2:40. Second occlusion of head arteries
2:40½. Rigidity almost entirely disappeared from fore limb
2:41. Head arteries released
2:42. Rigidity reappears
2:47. Corneal reflex present and limbs very rigid
2:49. Third occlusion of head arteries
2:49½. Rigidity disappears; corneal still present
2:50. Release of head arteries; corneal gone
2:51½. Rigidity returns
2:56. Good corneal
2:58. Respiration failing; no corneal and little rigidity
2:59½. Fourth occlusion; both magnitude and duration of anemic rise much less than before
3:00. Head arteries released
3:01. Rigidity in fore limb has not reappeared; hind limb stil rigid
3:10. No further rise in pressure; no return of respiration, decerebrate rigidity or corneal reflex

That the peripheral cardio-vascular mechanism was still intact at the conclusion of the above experiment, was shown by a rise of blood pressure from 70 mm. Hg to 140 mm. on compression of the abdominal aorta at this time. Had there been failure of the heart, it would have been impossible to obtain such a rise in pressure. The failure, then, was central.

The condition of the fore limb during these successive periods is shown by the drawing which was made at the time, the limb being allowed to assume its own position, with the cat lying on its back.

During the course of these experiments, it was also observed that the character of the respiration became almost wholly diaphragmatic after decerebration. There was little or no costal respiration. It has been shown by Pike and Coombs (1922) that the central connections of the afferent nerves from the intercostal and other muscles involved in the respiratory movements lie at least as high as the inferior colliculi. The

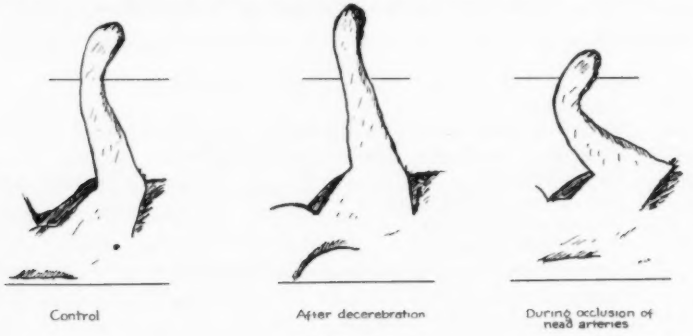

Fig. 1

disappearance of costal respiration during occlusion of the head arteries and its tardy return after release is merely another example of a rupture of central connections by anemia rather than trauma.

We wish to thank Prof. F. H. Pike of Columbia University for his kindness in criticising this work.

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THE EFFECT OF HISTAMINE ON CEREBROSPINAL FLUID PRESSURE

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A dilatation of the blood capillaries has generally been accepted as one of the cardinal characteristics of histamine action. For that reason plethysmographic studies on various organs and structures have demonstrated an increase in the volume after histamine had been given. Dale and Richards (1918–19) and also Burn (1922) observed that the extremity of a dog increased in size after an injection of this drug. However, Edwards (1920) has intimated that there may be a difference in the organs of the body regarding a dilatation of their blood capillaries. But if the dilatation were present throughout all organs of the body, then an increase in brain volume and an increased cerebrospinal fluid pressure might be expected, provided the arterial and venous volumes remained the same. For the skull, according to the Monro-Kellie doctrine (1783), (1824), may be considered as a physiologically closed box, particularly in view of the better evidence recently offered by Weed and Hughson (1921). Using the skull, then, as a plethysmographic chamber, some of the following evidence regarding pressure and volume changes within this rigid container were obtained. In short, instead of finding an increase in the cerebrospinal fluid pressure, an actual decrease was observed.

Experiments. The experiments were performed on ten cats and on an equal number of small dogs. All the animals were apparently healthy and showed no gross pathological lesions at necropsy. Ether, which was employed as the anesthetic throughout, was given by intratracheal insufflation. After a little experience the mixture of air and ether vapor was fixed at the beginning of the experiment, and no changes were subsequently made, even though some observations were extended over a period of two hours or more. Arterial blood pressure was obtained from the femoral artery and recorded on a kymograph; the common carotid artery was deliberately avoided because of its obvious close connection with the cerebral blood supply. The systemic venous pressure was determined from the brachial vein, using Ringer's solution as the manometric fluid. The pressure of the cerebrospinal fluid, as well as of the blood in the superior sagittal sinus, was measured according to methods

previously described by Weed and Hughson (1921). The histamine used in all these experiments was a preparation called Imido "Roche."[1] It was always freshly prepared in Ringer's solution and given intravenously in quantities of usually 2 cc. but never exceeding 5 cc. As a control, equal amounts of Ringer's solutions were frequently given intravenously, but no observable changes occurred. Furthermore, the administration was rapid, rarely requiring more than seven seconds.

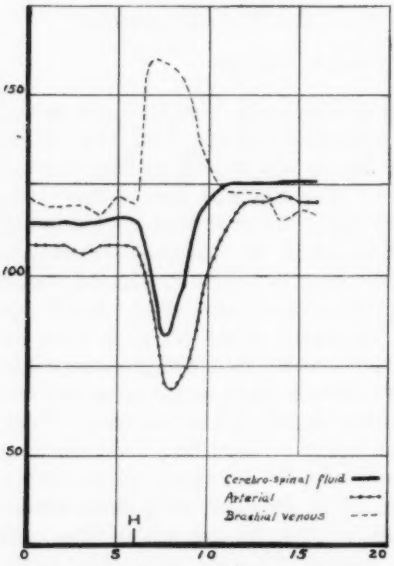
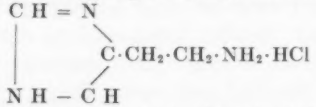


Fig. 1. To show the effect of an intravenous injection of 0.05 mgm. of histamine on an adult cat weighing 2650 grams. The ordinates represent millimeters of mercury for the arterial pressure, and millimeters of Ringer's solution for all other pressures; the abscissae denote the time interval in minutes. The injection was made at *H*.

The outstanding effect was a fall in the cerebrospinal fluid pressure. This result was produced by doses varying from 0.02 to 2 mgm. per kilogram of body weight. When relatively small quantities, 0.002 mgm. per kilogram of body weight, were injected, it occasionally happened that the usual fall was quickly followed by a smaller rise; in two instances a slight rise was observed, but it is believed that an unusually high general venous pressure was greatly responsible for this deviation from the customary effect.

In figure 1 the changes caused by 0.05 mgm. of histamine given to a cat weighing 2650 grams are charted. This dose was relatively small. It will be seen that the cerebrospinal fluid pressure fell sharply, reaching its lowest values about fifty seconds after the injection; then it rose steadily to a level slightly higher than the original one. The arterial blood

[1] This is the trade name of the Hoffman-La Roche Chemical Works and designates a substance having the following structural formula:



It is histamine hydrochloride, being the hydrochloride of the base beta-imidazolylethylamine, made by the elimination of one molecule of carbon dioxide from histidine to which it is closely related.

pressure paralleled the cerebrospinal fluid pressure very closely, reaching its lowest level sixty seconds after the injection, and subsequently rising above its initial values. The general venous pressure rose quickly and markedly, returning later to its former values; figuratively, it resembled the mirror image of the arterial blood pressure.

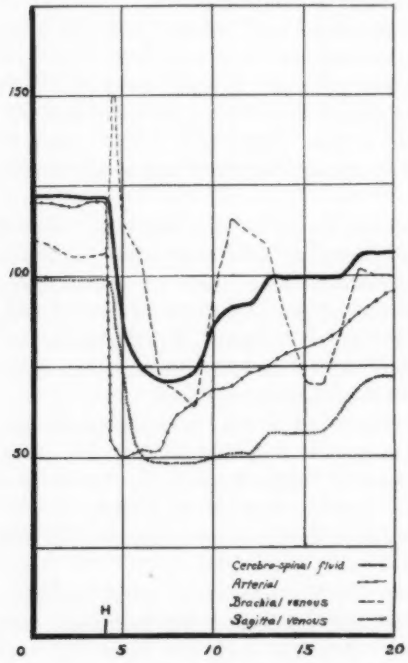


Fig. 2

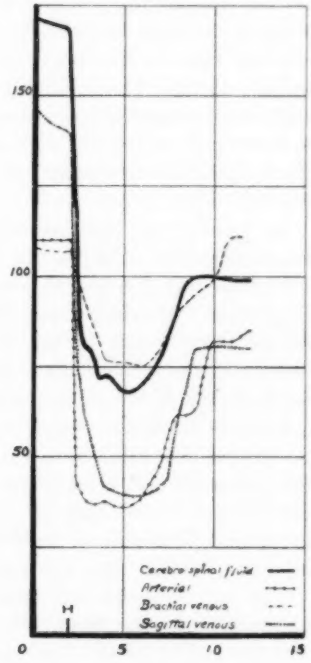


Fig. 3

Fig. 2. To show the effect of an intravenous injection of 0.5 mgm. of histamine on an adult dog weighing 4.5 kilograms. The ordinates represent the arterial pressure in millimeters of mercury and all the other pressures in millimeters of Ringer's solution; the abscissae indicate minutes. The injection was given at *H*.

Fig. 3. To show the effect of an intravenous administration of 1.0 mgm. of histamine on an adult dog weighing 5 kilograms. The arterial pressure is indicated in millimeters of mercury, whereas all the other pressures are represented in millimeters of Ringer's solution; the abscissae denote minutes. The injection was made at *H*.

It was believed that valuable additional information might be had from a record of the sagittal venous pressure, particularly in view of the great divergencies between the systemic venous and the cerebrospinal fluid pressure. For that reason, this pressure was obtained in one cat and five dogs. It was found that it paralleled the cerebrospinal fluid very

closely, reflecting, nevertheless, the systemic venous pressure. In figure 2 the various pressure values are illustrated.

From this figure it will be seen that the dose of 0.5 mgm. which was a fair average for the doses of medium size, caused such a pronounced fall in the cerebrospinal fluid that recovery was not completely established by the end of twenty minutes. Its descent was slightly more precipitous than in the case of figure 1. The arterial blood pressure also fell more sharply than in the previous illustration, and also remained below its initial value. The systemic venous pressure reacted peculiarly. It registered a sudden very temporary rise, followed by a very marked fall; it then appeared in the form of two waves. The effect of the second of these declines was clearly reflected in the sagittal sinus and cerebrospinal fluid pressures.

In figure 3 are charted values obtained also from a dog that weighed practically the same as the one in figure 2. However, twice as much histamine was given. This animal showed the reaction to a moderately large dose. It will be seen that the chief point of difference was a fall, rather than a rise, in the general venous pressure. Furthermore, the decline in the cerebrospinal fluid and sagittal venous pressures was more marked than in the animals shown in the other two figures.

In order to observe how low the cerebrospinal fluid pressure would fall, 8 mgm. of histamine were injected into a cat weighing 3 kilograms. The fluid pressure fell from 100 to 33 mm. of Ringer's solution. In another cat 2 mgm. of the drug produced a decline from 130 to 20 mm. in nine minutes. This animal never rallied and died twenty minutes after the injection. In no case was a negative value obtained.

The two animals just mentioned also illustrated the great variation in reaction to the drug. Several factors influenced the reaction. One was the height of the fluid pressure before the drug was given. If this was high, 150 mm. or more, then the subsequent fall was very decided; if it was below 100 mm. then the decline was relatively not so great. Another factor was the repeated administration of the same dose; subsequent injections did not cause a proportionate lowering of the fluid pressure. Finally, the depth of anesthesia also played a rôle. In deep anesthesia, the fall in cerebrospinal fluid pressure was more marked than when the animal was in relatively light narcosis.

Since histamine produced a fall in cerebrospinal fluid pressure, presumptive evidence was brought for the view that the small vessels of the brain, particularly the capillaries, did not dilate and cause an increase in brain bulk and thus of cerebrospinal fluid pressure. But since a fall in arterial pressure was produced invariably by the drug, the possibility existed that the capillaries did dilate but that the effect of their increased size was counteracted to such a degree by the decrease in blood volume

which probably took place as the result of the decrease in arterial pressure, that the net result was a decrease in the cerebrospinal fluid pressure.

Obviously, it was desirable to observe the small vessels of the brain directly, for then any increase in size after the administration of histamine might be detected. Accordingly, the skull was trephined over the parietal boss. A circular disc of the dura was carefully removed, and the surface of the brain was observed with a binocular microscope having a calculated actual magnification of 62.4 diameters. While the cortex was still covered with the cerebrospinal fluid, histamine in doses large enough to insure a characteristic effect as evidenced by kymographic arterial blood pressure records taken simultaneously, was injected intravenously. In neither of the two dogs in which this experiments was repeatedly tried did an appreciable enlargement of the small vessels take place. One observer noted a slight contraction of an arteriole.

It was also noticed in these animals that whereas the brain ordinarily eventually occluded the trephine opening, after histamine had been given the brain retracted from the wall of the skull, and the cerebrospinal fluid no longer covered the surface, but made the cortex appear dry and wrinkled. The disappearance of the fluid can be explained as being due to the draining of the fluid into the spaces made available by the decrease in the size of the brain. The atmospheric pressure obviously was also responsible for the volume changes.

However, in order to study the cortex under more physiological conditions, a circular piece of glass, 1.5 cm. in diameter, was mounted in a short steel cylinder and screwed into the trephine opening. This piece of apparatus served as a brain window, and was in some respects like the device previously employed by Donders (1851). Furthermore, it was watertight. Cerebrospinal fluid pressure determinations were made throughout the period of observation. In these four dogs also, in whom repeated injections were made, no appreciable change in the size of the smallest blood vessels was noticed. The definite fall in the fluid pressure, in one case from 75 mm. to zero, and later from 50 mm. to -5 mm. testified to the action of the drug as well as to the water-tight condition of the brain window. But even under these improved conditions, and after repeated injection of histamine, no engorgement of the small vessels on the cortex was observed.

Nevertheless, it might be said that the removal of the dura could have disturbed the subarachnoid space and pia mater sufficiently to affect the small vessels in the surface of the cortex. In order to bring histological evidence, Bouin's fluid was injected under the dura when the cerebrospinal fluid pressure had fallen very markedly; in one cat fifteen seconds and in the other one minute elapsed after histamine had been given. The injection was made in such a manner that the fixing fluid would flow over

the surface of the brain and escape through a puncture of the dura made as far away as possible from the point where the needle for the injection was inserted. The same experiment was repeated in two dogs; in one of these animals, fixation was done thirty seconds after the administration of the drug. Material for a control was taken from a cat and a dog in a similar manner. Subsequent histological examination of the imbedded material as well as of cleared specimens failed to reveal any difference in the size or engorgement of the vessels from animals which were injected with histamine and those that were not.

In short, all the evidence, whether it was from a study of the pressure changes in the fluid systems, whether it was from an examination of the cortex of the brain directly or with the aid of a brain window, or finally, whether it was from a consideration of histological material, indicated that the small vessels on the surface of the brain did not dilate after histamine had been given intravenously.

Discussion. As far as could be gathered from the literature, no direct observations of the effect of histamine on cerebrospinal fluid pressure have been recorded. In connection with their studies on the secretion of the cerebrospinal fluid, Dixon and Halliburton (1913) noted a sucking back of fluid in the cisternal puncture needle followed by an "apparent increased secretion" after histamine had been given. Obviously, the decreased cerebrospinal fluid pressure accounted for the sucking back, but it is more difficult to explain the subsequent increased flow unless it were due to the rise in arterial blood pressure which followed later.

Becht and Matill (1920) have reported the action of solutions of peptone which is similar to histamine in some of its effects (Dale and Laidlaw, 1910). They found that peptone decreased the fluid pressure on an average of 15.8 mm. However, this average did not reflect several marked variations; in one animal the pressure even rose 45 mm.

The evidence presented in these experiments indicated that the capillaries of the brain did not react to histamine, if in the same way at least not to the same degree as certain capillaries elsewhere in the body. There is no doubt about the reaction of the capillaires in the viscera, for Dale (1920) and his associates, Laidlaw (1918–19) and Richards (1918–19) described the congestion of the blood capillaries and venules in the abdominal viscera following the injection of histamine. Krogh (1922) has explained the increase in the depth of red color in striated muscle after the administration of this drug as being due to a capillary dilatation.

Although the skull was an ideal plethysmograph, there were several factors besides the size of the capillaries which could have reduced the cerebrospinal fluid pressure. In the first place, histamine might have suddenly decreased the production of the fluid or increased its transport into the venous system; in either case its pressure would have been less,

provided the other conditions remained the same. But, according to Weed (1922), our present knowledge of the amount of production and absorption of this fluid is still so imperfect that no conclusions regarding volume changes in it can be entertained.

Obviously, the fall in arterial blood pressure might explain the whole phenomenon, particularly since the curve of the fluid pressure and arterial were so often parallel. But it must not be forgotten that the fluid pressure did not always fall when the arterial pressure did. In one case, the fluid pressure did not change although the arterial pressure decreased even though slightly; and in another case the fluid pressure actually showed a slight temporary rise. However, it must be added that in this last instance, a remarkably high general venous pressure was registered, and it would not be unreasonable to assume that any volume loss which the low arterial pressure may have entailed was more than compensated for by a correspondingly high venous pressure (Hill, 1896). Finally, the drug might have decreased the brain bulk and thus produced a lower fliud pressure; but the speed of the reaction would argue against this view.

Briefly, it is difficult to explain away entirely the fall in the cerebrospinal fluid pressure on the basis of a decrease in the arterial pressure, but it is believed that the arterial pressure change was mainly responsible. But in the last analysis, the action of histamine is still so imperfectly understood (Krogh, 1922; McDowall, 1923) that a proper consideration of all the influences in this particular problem is impossible.

Again, the evidence presented here supported the view (Hill, 1896) that the blood supply to the brain could be influenced to such a degree by vasomotor changes in the viscera, that it was not necessary to ascribe vasomotor functions to the nerves which have been demonstrated on the cerebral vessels (Obersteiner, 1897; Stöhr, 1924). It is not the purpose of this article to debate the question of vasomotor nerves of the brain. It is sufficient to point out that Ackermann (1858), Biedl and Reiner (1900), Jensen (1964), Pick (1899), Weber (1909) and Wiechowski (1902) believed that there was a vasomotor mechanism, whereas Bayliss and Hill (1895), Hürthle (1889), and Roy and Sherrington (1890) opposed this view. Bayliss (1923), in his recent little book on the vasomotor system, stated that the vasomotor nature of these nerve fibers has not been sufficiently demonstrated

Furthermore, it may be argued that since the pia mater has characteristically very few small blood vessels, inspection of the brain was limited almost entirely to venules and not to capillaries. However, it may be said that engorgement of the capillaries would naturally have caused a marked filling of the small veins, so that, even though only a few capillaries were seen, the condition of these vessels was reflected in the size of the small venules.

It is also necessary to point out that the measures necessary to expose the cortex might have influenced the small superficial vessels to such an extent that they did not react characteristically. Rich (1921) has described how the handling of the omentum was sufficient to prevent the dilatation of the capillaries in histamine shock. It is believed that the direct fixation of the cortex counteracted to some extent this possibly unfavorable influence.

Many investigators have employed histamine to aid them in obtaining complete vascular injections. But a favorable result has not been invariable. Doan, Cunningham and Sabin employed histamine when they injected the inter-sinusoidal capillaries of bone marrow. They were unable to obtain any definite benefit from the use of the drug. In explanation, it may be ventured that the capillaries of bone marrow resemble those of the brain in that they are encased in a rigid container which may be also a physiologically closed structure (Drinker, Drinker and Lund, 1922).

Finally, Florey (1925) described recently the behavior of the blood vessels visible on the cerebral cortex of cats, rabbits and of a monkey. He showed that the arteries reacted to mechanical, thermal, electrical and chemical stimuli by contraction and dilatation. Furthermore, the "arterial ends of the capillaries" also showed this capacity to contract, whereas the "capillaries flowing into the veins" did not exhibit this reactivity. Although this investigator did not study the effect of histamine, nor institute many physiologically controlled experiments, it is interesting to note that he found the "capillaries flowing into the veins" passive in their reaction to many drugs.

SUMMARY

Histamine produced a definite fall in the cerebrospinal fluid pressure in cats and dogs under ether anesthesia. Although the blood capillaries of the viscera have been shown to become markedly dilated in histamine shock, all the evidence presented here indicated that the capillaries of the brain did not react in this way. These conclusions were based on: *1*, a study of the arterial, systemic venous, sagittal sinus, and cerebrospinal fluid pressures; *2*, direct observations of the small vessels on the cortex of the brain with and without the use of a water-tight brain window; and *3*, histological examination of cortical tissue fixed when the animal was in histamine shock.

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A NOTE ON DIFFERENCES OF CAPILLARY ACTIVITY

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The purpose of this article is to call attention to the marked differences exhibited by some of the blood capillaries. Since the blood vascular endothelium is so manifestly different histologically in the various organs of the body, there is no reason to expect it to be functionally the same, and once the endothelium has been granted physiological differences then there is no end to which this differentiation may be extended. Desirable as it is to draw upon physical and chemical forces for the explanation of fluid exchange, it is nevertheless impossible to escape the belief that the individual endothelial cells have the ability to regulate to some degree the transport of substances through their cell wall and cytoplasm. As an example, the formation of lymph has frequently been considered as a product of endothelial activity.

And taking the formation of lymph as an instance, it will be remembered that an increase in the blood capillary pressure alone has frequently been relied upon to explain this phenomenon. On this basis Starling (1894) has answered many of the objections raised by Heidenhain (1891) but even Starling was forced to resort to an increased permeability of capillaries in order to help explain the action of certain tissue extracts designated as lymphagogues of the first class. It may not be amiss to bring in a modified form (fig. 1), the diagram which Starling used to explain the action of crystalloids grouped as lymphagogues of the second class.

It will be seen from this figure that arrow *1* indicates the direction of flow from the tissue spaces to the blood capillary because of the increased osmotic pressure following the introduction of an hypertonic solution into the blood stream. The sudden hydremic plethora thus produced gives rise to an increased capillary pressure sufficiently high to cause a return flow to the tissue spaces as shown by arrow *2*. A part of the relatively large amount of fluid which has thus collected in the tissue spaces escapes into the nearby lymph capillary as indicated by arrow *3*. Therefore, the net result of the injection of an hypertonic solution into the blood stream was to cause an increased lymph flow.[1] But even in an explanation

[1] It seems advisable to adhere to a definite nomenclature of the lymphatic system, and restrict the term "lymph" to the fluid within lymph vessels, using the term "tissue fluid" to designate the liquid in the tissue spaces, and reserving ex-

supported mainly by the physical laws of filtration and osmosis, Starling was forced to rely upon an altered condition of the capillary wall. He spoke of the endothelium, saying at that time that it "is imperfect—it contains pores, through which fluid will transude under a certain pressure." From these considerations it can be understood why an intravenous injection of hypertonic salt solution may cause an increase both in lymph flow (Cohnstein, 1895) and in lymph pressure (Beck, 1924).

The discussion so far has taken into consideration only tissues having a lymph drainage. But certain organs, like the central nervous system, have no lymph vessels and it does not necessarily follow that the same type of reaction takes place in their blood capillaries, even though the cerebrospinal fluid system is in several particulars like the lymphatic system. Weed and his associates (1919), (1921) have demonstrated beyond doubt that the intravenous injection of an hypertonic salt solution will cause a decrease in the cerebrospinal fluid pressure as well as in the brain bulk. Now, if the brain capillaries react like the capillaries of the viscera, then an exchange would also take place as represented by arrow *2* in figure 1. But this reaction did not take place; the fluid transport was as indicated by arrow *1*. In short, there was apparently a fundamental difference between the reaction of the capillaries in the brain in contrast to those of the viscera.

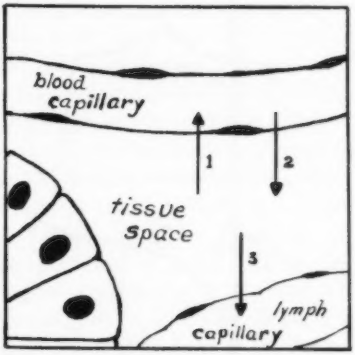

Fig. 1

In order to visualize this difference in capillary activity, and to combine in one procedure the results obtained by other investigators in different fields, the following experiment was performed on an adult cat, weighing 2365 grams. In this animal as in all the subsequent experiments the systemic venous pressure was measured in the brachial vein in millimeters of Ringer's solution; the cerebrospinal fluid pressure was determined according to the method of Weed and McKibben (1919); and the lymph flow was gauged by the number of drops which came from a short cannula inserted into the thoracic duct at the base of the neck. Seven and a half cubic centimeters of a 30 per cent sodium chloride solution were injected into the brachial vein during a period of four minutes, and it was

pressions like "peritoneal fluid" and "cerebrospinal fluid" for liquid in definite and specialized cavities. For although lymphatic endothelium is very permeable, it is nevertheless able to exclude certain elements, particularly morphological ones, and it can be considered as a differentiating membrane.

found that the cerebrospinal fluid pressure which was at 79 mm. had risen to 108 mm. by the time that the injection was completed and then fell to 10 mm. at the end of twenty-four minutes. The lymph flow, which was at the rate of 3 drops per minute at the beginning subsided completely during the salt injection, but subsequently rose to 9 drops; thirty-two minutes later it returned to its original rate of flow.

On the basis that a dilatation of blood capillaries is necessary for the production of lymph it was to be expected, as Dale and Laidlaw (1910) have shown, that histamine should cause an increased lymph flow. Beck (1924) has furthermore demonstrated that an increase in lymph pressure

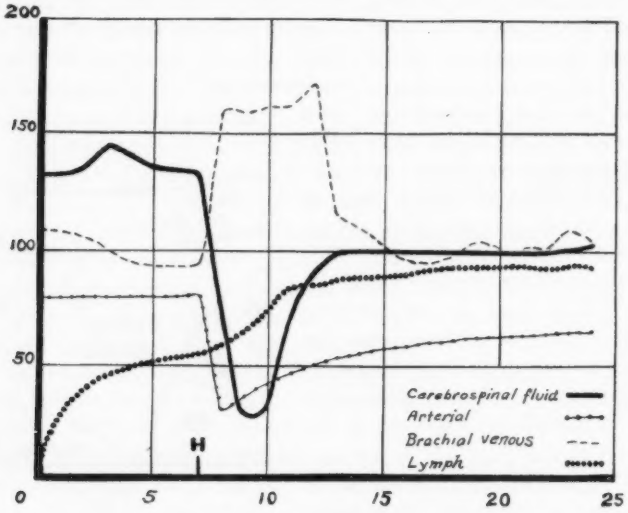

Fig. 2. To show the effect of the intravenous injection at *H* of 0.5 mgm. of histamine into a dog weighing 4.6 kilograms. Ordinates represent millimeters of mercury for the arterial pressure, and millimeters of Ringer's solution for all other pressures. The abscissae denote minutes.

also takes place. But if the dilatation of blood capillaries after the injection of histamine is general, then an increase in the brain bulk and in the cerebrospinal fluid pressure might be expected, provided the arterial and venous volumes remain the same. However, the reverse has been found to be true (Lee, 1925).

In order to represent graphically this difference in action of the blood vascular system in different parts of the body the curves in figure 2 are presented. It will be seen from this chart that the cerebrospinal fluid pressure fell sharply after histamine had been given, but that the lymph pressure, as determined in the thoracic duct according to a method pre-

viously described (Lee, 1924), immediately rose, slowly at first but more quickly after a minute had elasped, and eventually it reached a level which previous experience has taught would not have ordinarily been attained. The pressure changes in the femoral artery and in the brachial vein were essentially the same as previously recorded.

Another example is therefore offered to demonstrate the difference in capillary action. In many respects the action of an hypertonic salt solution and of histamine was the same in this series of animals. Both produced a fall in the arterial and cerebrospinal fluid pressures, and both caused a rise in lymph flow and in lymph pressure. But whether their mode of action was the same is doubtful. Osmotic forces may be called upon to explain the action of the salt solution; but in the case of histamine one must reluctantly fall back upon changes in the blood capillary endothelium.

This difference in the action of two substances was shown for instance in the remarkable differences in degree in the lowering of the cerebrospinal fluid pressure. In the case of the hypertonic salt solution negative pressures have been readily obtained (Weed et al., 1919, 1921); however, large doses of histamine killed the animal before such low pressures were recorded.

Furthermore, the method of administering the salt solution influenced the reaction. Ordinarily in animals the 30 per cent solution had been given intravenously in amounts varying from 4 to 20 cc. during a time interval of about 4 minutes (Weed and Hughson, 1921). However, it was noticed that if a small amount of the solution be given quickly an atypical reaction might be observed. As an illustration 1 cc. of a 30 per cent sodium chloride solution was injected within 3 seconds into the vein of a cat weighing 3160 grams. The fluid pressure promptly rose from 163 to 182 mm. of Ringer's solution and then receded to its initial level. The arterial pressure at first fell but then it also returned to its former level. The striking feature of this experiment was the increase in the fluid pressure with a return to values no lower than the original ones. In this case a true rise, rather than the customary decline in pressure occurred. A few minutes later another cubic centimeter of the salt solution was injected. In this case the fluid pressure also rose, but it fell to a point lower than the level preceding the injection. The arterial pressure suffered a temporary decline. Apparently the salt was beginning to demonstrate its characteristic effect, and therefore a larger dose would be necessary to obtain another rise in the fluid pressure. Accordingly, two cubic centimeters were given and the fluid pressure rose promptly but fell very much lower than before. The arterial pressure reacted as it did in the first instance, and as can be seen from figure 3, it gradually reached higher values after each salt administration. The same general results as found in this cat were also obtained in a small dog.

These experiments showed that only a rise, even though it be small, occurred in the cerebrospinal fluid pressure after the first administration of the salt. In almost every chart given by Weed and Hughson (1921) this rise in fluid pressure was progressive and occurred only while the salt was being administered.

One may venture to apply Starling's explanation as given in figure 1 and believe that the sudden injection of the hypertonic salt solution increased the molecular concentration in the blood stream and thus augmented the fluid in the blood vessels. This increase in blood volume was

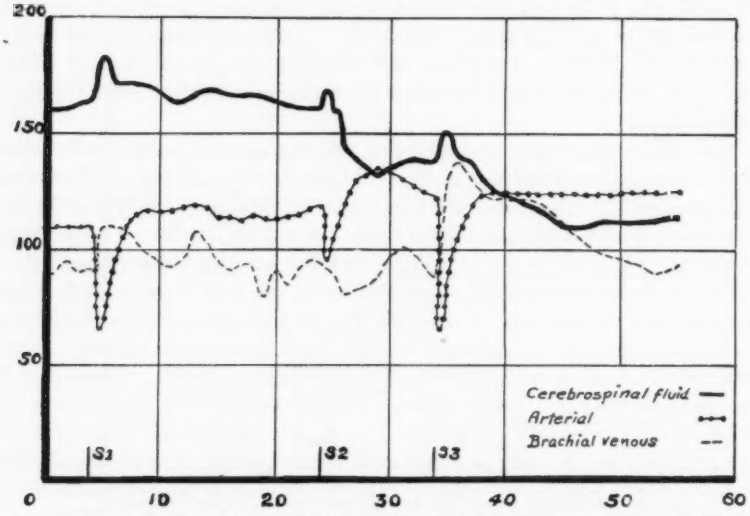

Fig. 3. To show the effect of the repeated administration of small quantities of a 30 per cent sodium chloride solution into a cat weighing 3.2 kilograms. At *S1* and *S2*, 1 cc. of this solution was given quickly intravenously; at *S3*, 2 cc. were injected. Ordinates represent arterial pressure in millimeters of mercury, whereas the other pressures are given in millimeters of Ringer's solution. Time intervals in minutes are recorded along the abscissae.

accompanied by an increase in the cerebrospinal fluid pressure. But the relatively high salt concentration in the blood was soon balanced by the transfer of salt from the blood vessels to the tissue spaces. Now it may be supposed that this exchange took place more readily in the viscera than in the brain, and that therefore the tissue spaces became distended and an increased lymph flow resulted. Furthermore, the salt concentration in the visceral tissue spaces was greater than in the brain tissue. And, therefore, while excretion of the salt was constantly taking place by means of the kidney, the fluid transport was partly from the brain to the visceral tissue by means of the blood stream.

It may also have been that the salt damaged the capillary endothelium and thus temporarily increased its permeability. However, this damage, if present at all, must have been very transitory, because the increase in the cerebrospinal fluid pressure persisted as long as the salt solution was being administered; once this injection was stopped the fluid pressure fell and the excretory mechanism of the body became active as was evidenced in part by the increased urinary output. While excretion was taking place, the salt content of the tissues which was suddenly raised to a high level also decreased. Therefore, any subsequent equal additions of salt to the blood stream influenced the reactions proportionately less.

That the action of hypotonic solutions (Weed and Hughson, 1921) differed radically from the hypertonic ones can be explained as being primarily due to the fact that the hypotonic injections increased absolutely the fluid volume of the whole body whereas the hypertonic solutions caused only a redistribution of the fluid already present.

Furthermore, one can believe that certain tissues are more adaptable to fluid exchanges than others. The central nervous system, for example, is composed of a very soft, fatty, more or less homogeneous substance, particularly free of gross intercellular spaces, enclosed within rigid walls, and poorly suited for fluid transport. On the other hand, the abdominal viscera, and particularly the retroperitoneal and subcutaneous spaces, since they have more areolar tissue, are much better better adapted for storing fluids temporarily. These "buffer spaces" have a certain flexibility and act as a protective mechanism for the body. Besides, the brain is located within a rigid container, and therefore well protected from gross volume changes. The same considerations are indicated also for the bone-marrow which is soft and fatty like brain tissue.

Whether the relative permeability of the blood capillaries is due to influences characteristic of the tissue in which the vessels lie is also by no means certain. Krogh (1922) has ventured to explain the presence of the large amount of calcium in cow's milk as due to the activity of the cells in the lactating gland which constantly remove the calcium from the perivascular tissues as fast as this crystalloid is transmitted by the capillary endothelium. But the difficulty consists in understanding why the endothelium allows particularly the calcium to go through.[2] However, this type of explanation is more difficult when applied to the renal glomeruli.

[2] Furthermore, there is no connection between the amount of calcium in the thoracic duct lymph and in the milk, and the age old problem, propounded by Heidenhain, regarding these amounts, has no place in the physiology of lymph formation.

The liver sinusoids may be considered also, since they have certain properties not shared by other capillaries. Their structure is still poorly understood anatomically (Bensley, 1923) and for that reason the following experiments may in the future meet with a simple explanation. It was noticed that when from 40 to 100 cc. of a mixture containing equal parts of India ink and salt solution were injected slowly into the brachial vein of a cat, the ink particles could be observed in the lymphatics at the hilum of the liver only at the end of a half-hour. This phenomenon was remarkable because the foreign particles must have passed not only through the sinusoidal but also the lymphatic endothelium. These results must be contrasted with the experiments of Krogh (1922) on the extremity of a frog. Krogh was unable to observe particles of India ink having a diameter of 200 micra micra pass through the blood capillary wall. Apparently, as Starling has suggested, the liver sinuses are more permeable than capillaries elsewhere.

Once it is granted that blood capillaries are different physiologically, and it seems that these vessels in the brain, liver and extremity do react differently, then one can also entertain the belief that the capillaries in every organ are adapted particularly for the function of the organ in question. Whether this adaptation is an inherent quality of the capillary endothelium or whether it is due to influences arising in the tissue around the vessel, or both, cannot be stated. It is obvious that explanations based, even though only partly, on known physical and chemical laws are preferable to views depending upon indefinite properties of cytoplasm; but unfortunately these laws do not completely suffice, and therefore an active participation of the endothelial cells must be considered.

SUMMARY

Evidence has been collected to point out the difference in the behavior of blood capillaries of various organs to the injection of sodium chloride, histamine, and India ink. The small vessels of the brain did not dilate like the visceral capillaries after histamine was given. Furthermore, they did not indicate the same fluid exchange with crystalloids as the capillaries in organs drained by the lymphatic system. Finally, the passage of particles of India ink from the blood to the lymph vessels of the liver indicated the high degree of permeability in the blood vascular bed of that organ.

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STUDIES ON MUSCULAR EXERCISE UNDER LOW BAROMETRIC PRESSURE

I. The Consumption of Oxygen and the Oxygen Debt

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We plan in a series of papers to consider the effects of a reduced barometric pressure upon the physiological responses of the body to muscular exercise. A reduction in the barometric pressure produces some disturbance of the biochemical condition in all persons. This is slowly compensated for if sufficient time, a day to a week or more, is spent under the new condition to permit the system to reëstablish an equilibrium. The experiments we are to report have been confined to short exposures to a lowered barometric pressure before there has been opportunity for these changes of acclimatization to occur. Heretofore almost no attention has been given to the study of muscular exercise during this early period.

All of our experiments have been conducted in a low pressure chamber at Mitchel Field (Obear, 1920) on young men who worked on the type of bicycle ergometer that has been described by Benedict and Cady (1912). Any degree of atmospheric pressure is easily obtained and maintained within the chamber. The chamber is connected with a mercury manometer which measures the pressure difference from the atmospheric barometric pressure. It is provided with a scale graduated in thousands of feet, based on the 20°C. isothermal altitude pressure tables of the U. S. Bureau of Standards. In all of the experiments the evacuation of the chamber was at a rate that simulated an altitude ascent of 1000 feet a minute, the decrease in pressure ranging from 27 to 11 mm. a minute, depending on the pressure. The load of work was also exactly prescribed and maintained.

The subjective effects of exercise at low barometric pressures were in no case alarming, although several of the men were rather timid about going to the lowest pressures. This feeling was relieved as their experience with a few experiments at moderate pressures produced no startling effects, as a result most of the men were willing to work at any pressure we desired.

In addition to the well known symptoms of anoxemia (Schneider, 1921) there were others due to the exercise itself. While sitting quietly on the

bicycle at pressures simulating an altitude of 15,000 to 25,000 feet a feeling of light-headedness and a disinclination to work was most pronounced, but when the work commenced the subject invariably felt better. At present it is not definitely known whether this is merely a subjective feeling or has a physiologic explanation in the respiratory and circulatory responses. During work the increased breathing was very noticeable at the lowest pressures. Often a feeling of tiredness and a desire to hold the breath, or at least to breathe more moderately, was present; but the air hunger at the same time kept urging to greater efforts in order to adequately ventilate the lungs. Occasionally a feeling of muscular weakness was noticed, and the subject would state that his legs "just wouldn't work;" but it was maintained that this was not the same feeling of exhaustion that ordinarily follows an intense effort. Headaches were not produced even by the most strenuous exercise. The cyanosis, which is so characteristic at pressures simulating 15,000 to 25,000 feet, was clearly present when the subject was at rest; but the color improved when pedalling commenced if the load was light. After work the dark blue flushed color of lips, ears and neck became even more pronounced than it was before work. In several instances the subject, when under the lowest pressures, showed the characteristic mental effects of lack of oxygen by becoming surly, stubborn or unmanageable. With the exception of one subject, who compensated poorly, none of the men showed signs of collapse.

The use of the bicycle in the low pressure chamber in respiration experiments very quickly showed the necessity of providing special respiration valves large enough to handle the greatly increased minute-volume of ventilation, which occasionally exceeded 100 liters a minute. The following arrangement was finally adopted and proved satisfactory. Two Douglas valves, with rubber flaps instead of mica disks, were used on the inspiration side; but in addition to the 24 holes which they originally had, 24 more were drilled in the seat plate of the valve, thus nearly doubling the capacity. Their combined effective area was 5.7 cm^2. Two of these valves were attached in a vertical position to the outer ends of an m-shaped assembly of three-quarter-inch iron pipe, and the mouthpiece was connected by a short piece of one-inch corrugated rubber tubing with the central pipe. The three arms of the (m) were not in one plane, those carrying the check valves being twisted away from the other to avoid contact with the subject's face. The whole was suspended from the ceiling and counterpoised so that the subject had to make no effort to keep it in position. From the lower side of the mouthpiece, a one inch rubber tube conducts the expired air to the expiratory valve. This was a Mueller water valve made from a large jar and one-inch brass pipe, with an effective area of 3.5 cm^2. The delivery pipe dipped about 2 mm. below the

surface of the water, and the resistance even with a high ventilation rate was slight. The bubbling was somewhat noticeable at first, but with every subject a few minutes, use accustomed him to it, enough for him to forget it entirely. From this valve another one-inch rubber tube was connected directly to the various bags in turn. The plan of set-up was similar to that used by Campbell, Douglas and Hobson (1920).

A kymograph was set up with a half-minute time marker, a signal magnet and key, and a tambour connected to the subject's side of the water valve. This record was used to verify the duration of each bag collection, and to count the rate of breathing, but of course not for the depth or character of breathing. Shortly before use each bag was flushed out with about 10 liters of expired air and emptied as completely as possible by a standardized method of rolling up and squeezing. A 10-liter Bohr meter

TABLE 1

The average oxygen consumption of H. M. during work, in cubic centimeters per minute

	TOTAL CONSUMPTION AT 760 MM. AND 0°C.					CORRECTED FOR THE INCREASE IN BREATHING				
	Sea level	10,000 feet	15,000 feet	20,000 feet	25,000 feet	Sea level	10,000 feet	15,000 feet	20,000 feet	25,000 feet
Rest	282	247	268	255	307	282	247	263	245	265
2000 ft-lbs.	945	912	883	834	771	873	825	784	731	669
4000 ft-lbs.	1535	1493	1430	1365		1375	1332	1226	1125	
6000 ft-lbs.	1883	1845	1675	1505		1670	1579	1397	1223	

was used for measuring the volume of the bags after the experiment. The seven bags used in these experiments were tested occasionally for leaks; but as the samples for analysis were always taken into the Bailey bottles soon after the filling, no checks were made to see whether the composition of a sample was altered by being left in the bag. In most cases the return from a low pressure to the normal was made before there was time for changes in volume, but in the longer experiments the meter was taken into the chamber and the measuring done there. For reducing these volumes to standard conditions the logarithmic factors of Boothby and Sandiford (1920) were used, supplemented by a similar table computed by us to 250 mm.

The relation of oxygen consumption to the load of work. In view of the fact that ordinarily the consumption of oxygen by the body per minute varies almost directly with the work done, the relationship of these two factors has been considered with 5 subjects. The data were secured while the subjects did in succession 2000, 4000 and 6000 foot-pounds of work per minute on the bicycle ergometer at barometric pressures comparable to those of sea-level, 10,000, 15,000, 20,000 and 25,000 feet. Prior to begin-

ning work the subject sat at rest at the chosen barometric pressure for a period of at least 10 minutes, in order to allow some time for the blowing off of the excess of carbon dioxide that normally occurs early in a period of anoxemia. Between the work periods a rest of 20 minutes was allowed, after which the subject then did the next larger load. Only one set of observations was made a day. Several experiments were made on each subject on widely separated days at each of the pressures. The data that are to be given are the averaged results of a number of experiments with each load at a single barometric pressure.

The largest number of experiments was made on H. M. and special care was taken to have the data for him complete. His average consumption of oxygen for each load of work at each barometric pressure is given in table 1. The data are also given in graphic form in figure 1. Since the experiments were conducted on widely separated days the resting-metabolism of the various experiments could hardly be expected to be identical. Consequently, the low barometric pressure reduction in the gaseous metabolism of the body, that was described in a former paper from this laboratory (Schneider, Truesdell and Clarke, 1924), is not as clearly shown as when experiments for comparison are made on the same day. There were 12 rest experiments made on this person at sea-level in which the oxygen consumption ranged between 245 and 348 cc., average 282 cc. If for the experiments under a reduced barometric pressure a correction in the oxygen consumption is made at the rate of 5.5 cc. for each liter of increase in the minute-volume of breathing, then a reduction in the tissue consumption of oxygen during rest is indicated at each low barometric pressure. In the second part of table 1 the data for oxygen consumption of the resting period have been so corrected. The averages of the original uncorrected data show a lessened consumption of oxygen during rest at all pressures, except at that corresponding to an altitude of 25,000 feet. Only one experiment was made at this pressure, and the high intake of oxygen then obtained may have been the result of annoyance to the subject, as it was necessary to talk to and disturb him continually in order to keep him awake. An almost irresistible desire to sleep comes over one at such a low barometric pressure. However, when a correction is allowed for the increase in breathing at the barometric pressure of 25,000 feet, the oxygen intake averages 17 cc. less than at 760 mm. Hence it is evident that some reduction in oxygen consumption occurred during rest at the several low pressures studied, but the decrease was not proportional to that of the barometric pressure.

That a lowering of barometric pressure may result in a decrease in the amount of oxygen consumed becomes clearer when the consumption of oxygen is observed during physical work and especially with the heaviest load. The average total minute intake of oxygen by H. M. with a load of

2000 foot-pounds was 945 cc. at 760 mm. and 771 cc. at 290 mm. (equivalent to an altitude of 25,000 feet), or a reduction of 174 cc. The intake was reduced 33 cc. at a pressure that simulated 10,000 feet, 29 cc. more at 15,000 feet, another 49 cc. at 20,000 feet, and an additional 63 cc. at 25,000 feet. For a minute-load of 4000 foot-pounds the intake of oxygen was reduced 42 cc. at 10,000 feet, 63 cc. more at 15,000 feet, and 65 cc. more at

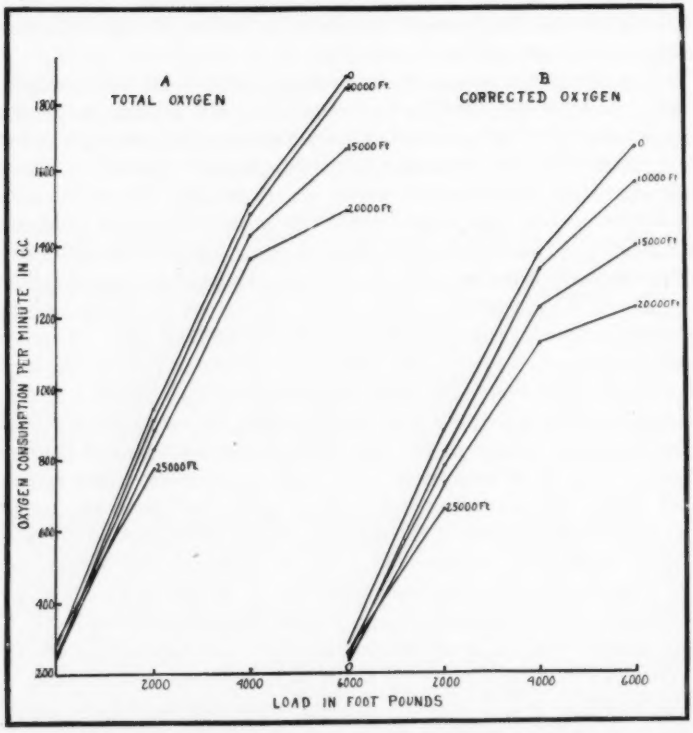

Fig. 1. *A*. The total amount of oxygen absorbed by H. M. with loads of work up to 6000 foot-pounds at various barometric pressures up to that which corresponds to an altitude of 25,000 feet. *B*. The amount of oxygen after a deduction has been made for the increased amount used by the respiratory muscles.

20,000 feet. It was deemed unwise to have this subject do 4000 foot-pounds of work at 25,000 feet. At 20,000 feet the intake of oxygen for this load was 170 cc. less per minute than at sea-level. With a load of 6000 foot-pounds the usage of oxygen fell off 38 cc. at 10,000 feet, 170 cc. more at 15,000 feet, and a further 170 cc. at 20,000 feet. Hence the total intake of oxygen was 378 cc. less per minute at 20,000 feet than at sea-level.

The reduction in oxygen usage at a barometric pressure of 375 mm. (20,000 feet) averaged 11.7 per cent for 2000 foot-pounds, 11.1 per cent for 4000 foot-pounds, and 20.1 per cent for 6000 foot-pounds.

At the several barometric pressures studied the absorption of oxygen by the body was approximately a linear function of the load of work up to that at which the load became an overload. This relationship is quite evident in the graphic illustration of our data given in figure 1. At sea-level, the linear function was maintained to, and including, a load of 4000 foot-pounds; but with a load of 6000 foot-pounds the consumption of oxygen was so decreased that it caused a slight deviation of the line. Under the influence of a reduced barometric pressure, the body more and more failed to provide the required oxygen for an overload of work. This is well shown in figure 1. It will be observed that there is a very pronounced bending of the lines downward with each decrement in barometric pressure for the load of 6000 foot-pounds, which was found to be an overload at the normal barometric pressure.

We have been especially interested in learning to what degree the anoxemia caused by a reduced barometric pressure affects the metabolism of the muscles of the body during work. In order to answer the question exactly as regards the amount of oxygen consumed by the muscles, a deduction should be made from the total amount of oxygen consumed for the additional oxygen used by the muscles of the heart and respiration. Both the frequency of heart beat and the minute-volume of breathing are augmented above their average during anoxemia. As has been pointed out (Krogh, 1916), the amount of oxygen consumed per heart beat has not been determined; but it is known that the amount of oxygen consumed by the muscles of respiration ranges, according to various authorities, from 5 to 10 cc. per liter of air respired. We have, therefore, following the work of Bornstein and v. Gartzen (1905), assumed that an additional amount of 5.5 cc. of oxygen is required for each liter of increase in the minute-volume of breathing caused by a reduction in the barometric pressure and by a heavier load of work. In the second part of table 1 such a correction of the data for oxygen consumption has been made. The corrected data are also plotted in *B* of figure 1. They merely accentuate the reduction in oxygen consumption and give the same, but emphasized, break in the altitude-oxygen consumption lines.

When the corrected data are studied, the differences between the amounts of oxygen consumed during a given load of work at sea-level and at the several barometric pressures show for all loads a linear relationship between the decrease in the amount of oxygen used by the active muscles and the reduction in barometric pressure. These differences have been plotted for the three loads, 2000, 4000 and 6000 foot-pounds, in figure 2. For H. M. they clearly establish a linear function between the

consumption of oxygen and the reduction in barometric pressure for each load of work. In other words, the amount of oxygen used by the muscles during work at any barometric pressure is rather exactly limited by the pressure of the available oxygen supply. The reduction in the amount of oxygen consumed per minute by the working muscles, other than those of respiration, for loads of 2000, 4000 and 6000 foot-pounds was at 10,000 feet 5.5, 3.1 and 5.4 per cent respectively; at 15,000 feet 10.2, 16.3 and 15.4 per cent respectively; and at 20,000 feet 16.2, 18.2 and 26.8 per cent respectively.

Our other subjects were not used in as many experiments of this type as was H. M. Hence a summary of their data does not reveal the same perfect relationship between oxygen consumption and decreased barometric pressure. On the whole, however, these support the data obtained with H. M. R. W. C. served as subject at least once at each reduction of pres-

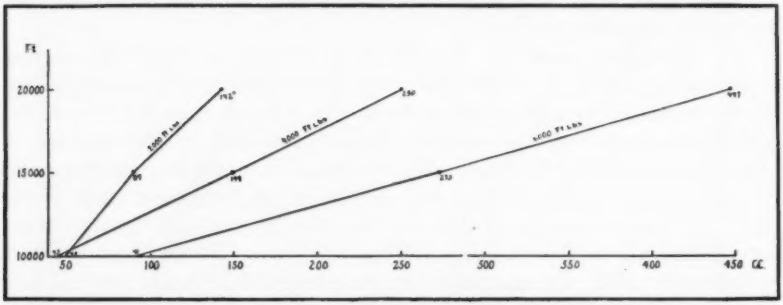

Fig. 2. Curves showing the linear character of the reduction in the intake of oxygen when work is done under low barometric pressures. The decrease in the number of cubic centimeters of oxygen consumed per minute for each load of work is plotted with respect to altitude.

sure in this type of experiment. These data for him are given in table 2, and have been corrected for the increased consumption of oxygen of augmented breathing. It is noteworthy that his total and corrected oxygen consumption were not affected during work at a barometric pressure of 10,000 feet for all loads of work up to and including 6000 foot-pounds. However, at that pressure that simulated an altitude of 10,000 feet when he carried a load of 8000 foot-pounds, he was unable to entirely meet the demand for oxygen; the consumption then fell off 297 cc., or 11.2 per cent. At 15,000 feet the body was unable to meet the full demand for oxygen under any of the loads, and at 20,000 feet it still less adequately met the needs.

The linear relationship between the decrease in the amount of oxygen consumed by the specially loaded muscles and the reduction in barometric

pressure cannot be established by these experiments on R. W. C. This is due to the fact that the reduction in barometric pressure equivalent to an altitude of 10,000 feet produced no change in the amount of oxygen consumed. Other irregularities, such as poor technique in conducting the experiments, may also account for some differences. The experiments on R. W. C. were among our earliest. The failure of the body to meet the oxygen requirements at 20,000 feet was neither proportional to the decrease in barometric pressure nor to the load of work. The drop in oxygen consumption by the specially loaded muscles, when at 20,000 feet, amounted to 10.2 per cent with a load of 2000 foot-pounds; 10.4 per cent with 4000 foot-pounds; and 30.2 per cent with 6000 foot-pounds.

A. L. H. served as a subject at 760 mm., 10,000 and 15,000 feet, carrying loads of work up to 8000 foot-pounds at an altitude of 10,000 feet, and 6000 foot-pounds at 15,000 feet. His corrected oxygen consumption per minute was practically unaltered by a decreased barometric pressure with a load of 2000 foot-pounds; but with a load of 4000 foot-pounds, it fell

TABLE 2

The oxygen consumption of R. W. C., during work, in cubic centimeters per minute

	TOTAL CONSUMPTION AT 760 MM. AND 0°C.				O_2 CORRECTED FOR INCREASE IN BREATHING			
	Sea level	10,000 feet	15,000 feet	20,000 feet	Sea level	10,000 feet	15,000 feet	20,000 feet
Rest	261	260	215	293	261	260	215	293
2000 ft-lbs.	829	846	795	767	776	780	727	697
4000 ft-lbs.	1355	1354	1273	1280	1203	1213	1140	1078
6000 ft-lbs.	1891	1914	1676	1545	1660	1683	1416	1124
8000 ft-lbs.	2661	2364			2377	2003		

off 58 cc. at 10,000 feet and 227 cc. at 15,000 feet; with a load of 6000 foot-pounds, it fell off 76 cc. at 10,000 feet and 488 cc. at 15,000 feet; with a load of 8000 foot-pounds, it fell off 895 cc. at 10,000 feet.

Another subject, C. R. J., was carried through with loads of work up to and including 8000 foot-pounds at 15,000 feet, 6000 foot-pounds at 20,000 feet, and 4000 foot-pounds at 25,000 feet. With a load of 2000 foot-pounds, his oxygen consumption remained practically unaltered at all barometric pressures up to that corresponding to an altitude of 20,000 feet, at which pressure it was reduced by 46 cc., 5.4 per cent; but at a pressure corresponding to 25,000 feet, it was reduced 124 cc., or 15.7 per cent. With a load of 4000 foot-pounds, the oxygen consumption of the specially loaded muscles fell off 25 cc. at a barometric pressure of 515 mm., 108 cc. at 425 mm., 268 cc. at 355 mm. and 585 cc. at 290 mm. (25,000 feet). The reduction at 290 mm. amounted to 41.7 per cent of the oxygen consumed by the specially worked muscles during this load at the normal barometric pressure. A load of 6000 foot-pounds was carried at 355 mm. (20,000

feet) with a reduction of 439 cc. in oxygen usage, or 27.4 per cent. A load of 8000 foot-pounds was carried at 425 mm. (15,000 feet) with a reduction of 318 cc., or 14.9 per cent, in oxygen consumption. This load of work was not done at the lower barometric pressures because of the distress it caused the subject.

Data secured from S. S. Y. are too incomplete to add anything more to this series of observations. He experienced some reduction in oxygen consumption with all loads of work at all the low barometric pressures to which he was exposed. The reduction was at first approximately linear; but with 4000 foot-pounds, it became excessive at the pressure which corresponded to an altitude of 20,000 feet. While with a load of 6000 foot-pounds, the reduction, at a pressure that simulated an altitude of 10,000 feet, amounted to only 10.5 per cent; yet the condition of the subject was such that it was deemed inadvisable to try a heavier load.

Step experiments. In these experiments, after a resting determination of the respiratory exchange at sea-level, approximately 760 mm., the subject worked under the predetermined load for a period of 5 minutes, the expired air being collected during the last 2 minutes of work. When the pulse and blood pressure had returned to normal, the subject moved to a chair. The barometric pressure was then gradually lowered to simulate approximately an altitude of 10,000 feet. After 5 minutes at the new pressure, the subject again mounted the bicycle ergometer; and, exactly 10 minutes after reaching the new pressure, commenced work with the same load as was carried at sea-level. The work period and the collection of expired air were the same as at the higher pressure. The same routine was then repeated successively at the other low pressures.

The data for 3 pairs of experiments on 3 subjects have been tabulated in table 3. An advantage of this type of experiment is that it eliminates the variations of metabolism that occur from day to day. We had hoped that this method of approach would more clearly bring out the linear relationship between oxygen consumption and the decrease in barometric pressure, but it did not. These data give further clear support to the fact that a reduction in barometric pressure causes a fall in the rate of oxidation in the body. In these experiments R. W. C. showed a decrease in the total amount of oxygen used at a barometric pressure of 548 mm. (approximately equivalent to an altitude of 10,000 feet) with a load of 2000 foot-pounds and a further decrease at each succeeding downward step in pressure. In the other type of experiments on him, given in table 2, the decrease was not present at this pressure. It was present, however, in the step experiments at 548 mm. for both the 2000 and 4000 foot-pound loads; but with the 4000 foot-pound load, it requires that the correction of the data for the augmentation of breathing be made in order to make this clear. It appears from the corrected data that at 548 mm. the use of oxygen by the loaded

TABLE 3

Step experiments in the low pressure chamber. Respiratory exchange during the 4th and 5th minutes of work

BAR. IN MILLIMETERS	MINUTE VOLUME IN LITERS	RESPIRATION RATE	EXHALED AIR		CO_2 IN CUBIC CENTIMETERS	O_2 IN CUBIC CENTIMETERS	R.Q.
			CO_2 per cent	O_2 per cent			
R. W. C.							
Work, 2000 foot-pounds a minute							
776	18.1	16.0	5.2	15.9	691	867	0.80
548	20.0	16.5	6.7	14.6	703	844	0.83
463	22.0	18.0	6.8	14.5	697	776	0.90
391	23.9	17.5	7.1	14.2	705	745	0.95
Work, 4000 foot-pounds a minute							
760	32.5	21.5	4.2	16.2	1250	1440	0.87
536	38.5	26.5	5.0	15.1	1199	1437	0.83
450	44.4	28.5	6.0	15.1	1400	1350	1.03
385	53.9	34.5	6.1	15.5	1440	1270	1.13
E. W. G.							
Work, 2000 foot-pounds a minute							
760	19.0	13.5	5.1	15.9	765	860	0.89
532	19.6	14.0	7.1	14.0	743	853	0.87
447	20.8	14.0	7.6	13.4	782	818	0.96
375	26.7	18.0	7.1	13.8	898	813	1.11
Work, 4000 foot-pounds a minute							
750	33.7	17.0	4.6	16.0	1597	1392	1.15
522	35.6	18.0	6.3	14.5	1470	1393	1.06
437	38.5	18.5	6.4	14.6	1459	1277	1.14
365	52.8	20.7	5.9	14.8	1661	1321	1.26
H. M.							
Work, 2000 foot-pounds a minute							
758	23.1	25.0	4.2	16.2	845	961	0.88
530	23.6	24.5	6.0	14.5	848	936	0.91
445	25.8	27.0	6.4	14.0	807	928	0.87
373	27.5	28.0	7.2	13.7	794	866	0.92
Work, 6000 foot-pounds a minute							
769	45.9	29.0	4.9	16.2	1982	1888	1.05
541	52.3	34.0	6.2	15.3	1929	1780	1.08
456	55.9	35.0	6.6	15.3	1875	1654	1.13
384	53.1	35.0	7.3	14.8	1629	1427	1.14

muscles was decreased 33 cc. with a load of 2000 foot-pounds and 36 cc. with a load of 4000 foot-pounds per minute. At the lower barometric

pressures the oxidation was still further reduced and the amount of reduction was approximately in linear relationship to the reduction in pressure. The oxidation in the specially loaded muscles fell off, with a load of 2000 foot-pounds, 112 cc. at 463 mm. (approximately 15,000 feet) and 154 cc. at 391 mm. (approximately 20,000 feet); with a load of 4000 foot-pounds, it fell off 155 cc. at 463 mm. and 288 cc. at 391 mm.

The 2 step experiments on H. M. (see table 3) should be compared with our earlier data on him that are given in table 1. In both types of experiments he experienced an early reduction in the oxidation processes and the reduction was approximately a linear function of the decrease in barometric pressure. In the step experiment with a load of 2000 foot-pounds the oxygen consumption, when corrected for the increased effort of breathing, shows a drop of 28 cc. at 530 mm., 48 cc. at 445 mm., and 119 cc. at 373 mm. The linear relationship to barometric pressure was more perfect with the load of 6000 foot-pounds, in which the corrected data show a fall in oxygen usage of 143 cc. at 541 mm., of 289 cc. at 456 mm., and 501 cc. at 384 mm.

The step experiments on E. W. G. are of special interest because he showed but little or no drop in the rate of oxidation while at a pressure corresponding to an altitude of 10,000 feet. With loads of work of 2000 and 4000 foot-pounds there is just a suggestion in the corrected data that a barometric pressure of about 530 mm. is near the limit of reduction in pressure that can be tolerated without an influence on the oxidative processes. At 532 mm., approximately equivalent to 10,000 feet, the corrected data for oxygen consumption show a drop of only 10 cc. for both loads of work. In the succeeding steps the drop is well defined but does not show the linear relationship. For the corrected data, with a load of 2000 foot-pounds, his usage of oxygen by the loaded muscles fell off 52 cc. at 447 mm. and 89 cc. at 375 mm.; with a load of 4000 foot-pounds, it fell off 141 cc. at 437 mm. and 176 cc. at 365 mm.

From the two preceding types of experiments the conclusion seems warranted that a lowering of the barometric pressure causes a reduction in the rate of oxidation in the muscles of the body during exercise. The decrease in barometric pressure becomes effective in most men when the pressure has been lowered approximately 235 mm., equivalent to ascending to an altitude of 10,000 feet. The decrease in oxidation is to a degree proportionately affected by the size of the load, it is, therefore, more pronounced with heavy loads. The decrease in the rate of oxidation is in linear relation to the decrease in barometric pressure.

In the two previous series of experiments a single determination of the respiratory exchange was made during work. In the series now to be given the exchange was determined minute by minute during a period of eight minutes of work and in an after period of 22 minutes. From these data information becomes available not only regarding the influence of a

decreased barometric pressure upon the total amount of oxygen consumed, but also upon the steady state, "the oxygen debt," and the efficiency and speed of recovery.

Changes during the exercise period. Hill (1923) and his collaborators have demonstrated that it is possible for a man to take an amount of muscular exercise that requires far more oxygen than can be supplied during the exercise. This is due to the fact that oxygen is not used in the process of muscular contraction but afterward during the process of recovery. The muscle is capable of going into debt for oxygen. Hence the body can exert itself far more violently than would be possible were it obliged to obtain all the energy immediately by combustion. The muscle accumulates its energy in a form known as a lactic acid precursor, glycogen, which is easily and quickly made available at the instance of contraction. The energy becomes available by the transformation of glycogen into lactic acid and this process is immediately followed by a contraction. Afterward the lactic acid remains to be reconverted into glycogen or oxidized. In the reconversion process approximately one molecule of lactic acid is oxidized to every five reconverted. If after contraction enough oxygen is not available for reconverting the acid into precursor then the acid remains, so to speak, as a security for future oxidation. In other words, the amount of lactic acid is a measure of the oxygen debt.

According to the above theory a deficiency in the available supply of oxygen should cause the oxygen debt after a selected load of work to be larger than when the supply is abundant.

It seems reasonable to assume, in view of this theory, that when a given load of work is done under a low barometric pressure that the difference between the amount of oxygen consumed at sea-level and at the low barometric pressure should indicate the amount of increase in the oxygen debt. In all of our experiments at low pressures we find the consumption of oxygen was clearly reduced during each of the 8 minutes of work. Most of the available data are for a load of 4000 foot-pounds, but some data are also available for a load of 6000 foot-pounds. Our experiments have been made on 4 subjects at barometric pressures corresponding to altitudes of 15,000 and 18,000 feet. One disadvantage, as in our first series, was that the tests had to be made on different days; hence the resting respiratory exchange varied considerably. In spite of this the results are surprisingly definite. With a load of 4000 foot-pounds each of the four subjects reached practically a steady state at the several barometric pressures and this was true in spite of the fact that the consumption of oxygen was reduced during exposure to the low pressure.

The data for a set of 3 experiments with R. W. C. are given in table 4, and graphically in figure 3, in which a load of 4000 foot-pounds was carried steadily for 8 minutes at barometric pressures of 764, 447 and 415 mm.

It should be noted that when the exercise first started the oxygen intake immediately rose; but it did not reach its maximum, the "steady state," until the third minute at 764 and 447 mm. and until the fourth or fifth minute at 415 mm. However, thereafter the usage of oxygen was fairly well maintained on a level. For each minute the intake of oxygen was less at the low pressures than in the corresponding minute at a barometric pressure of 764 mm. The total intake of oxygen during the 8 minutes of

TABLE 4

Three respiratory exchange experiments

4000 foot-pounds. 8 minutes. Subject R. W. C.

TIME OF BAG	BAR. 764 MM.					BAR. 447 MM.					BAR. 415 MM.				
	Minute volume	Respiration rate	CO_2 in cubic centimeters	O_2 in cubic centimeters	R.Q.	Minute volume	Respiration rate	CO_2 in cubic centimeters	O_2 in cubic centimeters	R.Q.	Minute volume	Respiration rate	CO_2 in cubic centimeters	O_2 in cubic centimeters	R.Q.
Rest	7.1	13.3	190	233	0.817	7.1	13.8	176	219	0.804	6.8	16	169	181	0.930
Work															
1′	16.8	16.0	637	883	0.721	22.0	23.0	653	804	0.812	22.6	22.0	705	731	0.964
2′	27.2	16.3	1050	1331	0.789	33.1	20.7	1159	1168	0.993	37.5	22.8	1104	1095	1.008
3′	31.6	19.0	1300	1453	0.984	39.8	25.0	1408	1379	1.021	43.9	29.4	1312	1053	1.246
5′	33.3	22.0	1412	1465	0.964	42.4	27.0	1371	1313	1.045	47.2	33.0	1447	1240	1.168
7′	33.4	21.0	1394	1496	0.932	41.3	29.0	1417	1395	1.016	48.3	34.0	1315	1252	1.050
8′	34.7	20.7	1406	1456	0.966	38.9	26.1	1266	1328	0.954	50.0	34.8	1343	1260	1.067
Rest															
1′	23.5	17.4	771	760	1.014	25.2	20.7	613	791	0.776	31.2	26.0	916	836	1.095
2′	14.6	16.3	421	372	1.132	15.1	16.3	362	392	0.925	16.7	19.3	456	380	1.203
3– 4′	10.5	15.9	270	300	0.898	10.6	17.2	251	284	0.886					
5– 7′	8.5	14.9	230	261	0.879	8.2	13.7	224	261	0.857	8.8	15.4	213	233	0.915
8–10′	8.6	16.4	219	267	0.818	7.5	13.7	197	248	0.795	8.6	16.4	203	224	0.906
11–13′	8.1	14.0	207	235	0.880	6.7	12.3	157	220	0.716	8.9	15.4	220	278	0.791
15–17′	7.2	14.3	183	245	0.750	7.8	12.3	194	260	0.748	9.3	15.3	215	248	0.866
19–22′	7.4	13.0	185	229	0.806	7.4	13.0	184	234	0.786	8.4	14.5	188	218	0.860

work was 11,023 cc. at 764 mm., 10,088 cc. at 447 mm., and 9024 cc. at 415 mm. After deducting the amount of oxygen that would have been consumed during rest, the intake of oxygen used in work amounted to 9159 cc. at 764 mm., 8336 cc. at 447 mm., and 7576 cc. at 415 mm. From which we find that the intake of oxygen during 8 minutes of work was 824 cc. less at 447 mm., and 1583 cc. less at 415 mm. than at 764 mm. These two quantities represent, therefore, the expected increase in the oxygen debt at the low barometric pressures above that experienced at 764 mm.

Lupton (1922) has suggested that the "lag" in the usage of oxygen behind that of the maximum usage during the maintained level of activity should be equal to the "oxygen debt." The "lag" was present, of course, only during the first minutes of work that preceded the "steady state." From the "lag" it appears that the oxygen debt of R. W. C. was 721 cc. at 764 mm., 744 cc. at 447 mm., and 984 cc. at 415 mm. The results were arrived at by taking the average of the "steady state" determinations, multiplying by 8 and deducting the actual usage from this amount. By this means a moderate increase of 263 cc. in the oxygen debt is indicated at 415 mm., but this falls far under the expected increase of 1583 cc.

The data for our other subjects for the load of 4000 foot-pounds give similar results. Thus with H. M.—see table 5—the actual intake of oxygen during 8 minutes of work at 760 mm. was 11957 cc. and at 410 mm., 9624

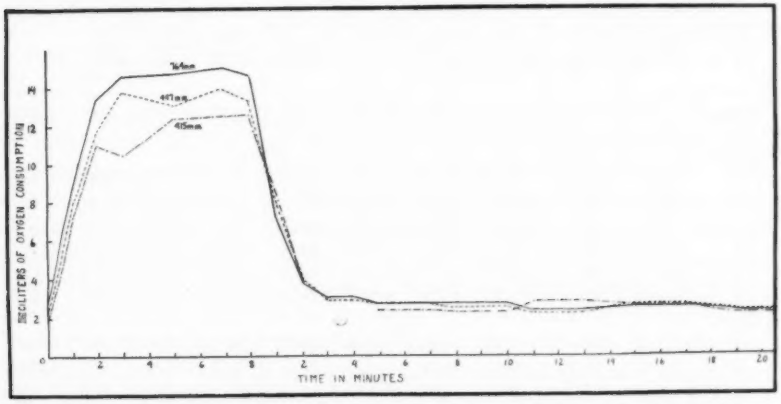

Fig. 3. Curves showing the intake of oxygen during and after a load of 4000 foot-pounds at pressures that simulated altitudes of sea-level, 15,000 and 18,000 feet.

cc. After deducting the amount that would have been used during rest, the work intake was found to be 9901 cc. and 7384 cc. respectively. The "oxygen lag" was 627 cc. at 760 mm. and 576 cc. at 410 mm. No increase, but rather a slight decrease, is thus indicated in the oxygen debt. Judging from the actual use of oxygen an increase of 2517 cc. was to be expected.

E. W. G. served as a subject in experiments at 756 mm., 457 mm. and 415 mm. At 756 mm. a "steady state" of oxygen usage was reached at about 1462 cc. At 457 mm. there was not a satisfactory "steady state," in that after reaching 1188 cc. the intake dropped to 1017 cc., but at 415 mm. a good "steady state" at approximately 1170 cc. was reached. The total consumption of oxygen in the 8 minutes of work was 10599 cc. at 756 mm., 8305 at 457 mm., and 8501 cc. at 415 mm. With the deduction

of the amount of oxygen used during rest the work intake of oxygen was 8295 cc., 6353 cc. and 6301 cc. respectively. It is evident, therefore, from the above data that an increase of 1942 cc. at 457 mm. and 1994 cc. at 415 mm. should occur in the oxygen debt. The "oxygen lag," supposed to be a measure of the oxygen debt, was 1097 cc. at 756 mm., 1167 cc. at 457 mm., and only 763 cc. at 415 mm.

In a pair of experiments on E. F. F. at 766 mm. and 457 mm. the oxygen intake during the 8 minutes of work, after correction for the rest consumption, was 1193 cc. less at the low pressure than at that of sea-level. This then is the expected increase in the "oxygen debt" at 457 mm. On the other hand the "oxygen lag," which was 1737 cc. at 766 mm. and only 752 cc. at 457 mm., did not conform to this expected increase.

TABLE 5

4000 foot-pounds for 8 minutes. Subject H. M.

TIME OF BAG	BAR. 760 MM.					BAR. 415 MM.				
	Minute volume	Respiration rate	CO_2 in cubic centimeters	O_2 in cubic centimeters	R.Q.	Minute volume	Respiration rate	CO_2 in cubic centimeters	O_2 in cubic centimeters	R.Q.
Rest	7.7	16.6	215	257	0.835	8.7	15.5	251	285	0.879
Work										
1′	20.7	24.0	740	983	0.735	21.9	27.0	731	800	0.914
2′	36.8	26.1	1506	1622	0.929	41.9	31.5	1377	1228	1.123
3′	37.6	27.2	1456	1515	0.961	45.7	33.7	1420	1228	1.156
5′	40.0	26.0	1571	1571	1.000	46.6	36.0	1437	1281	1.123
7′	40.5	29.0	1573	1577	0.998	46.9	34.0	1381	1274	1.084
8′	41.0	26.1	1538	1573	0.977	49.3	33.7	1494	1438	1.039
Rest										
1′	27.3	23.9	1178	1002	0.934	30.6	26.1	909	843	1.079
2′	13.9	20.7	402	350	1.149	19.4	22.0	501	470	1.067
3– 4′	9.7	18.2	253	289	0.872	11.5	21.3	288	337	0.856
5– 7′	11.1	18.8	301	321	0.940	10.5	18.7	239	288	0.829
8–10′	9.3	20.0	255	301	0.847	9.3	21.7	229	280	0.817
11–13′	9.7	17.5	266	310	0.859	9.6	19.9	228	289	0.789
15–17′	7.0	15.3	197	247	0.799	9.0	14.3	217	287	0.758
19–22′	7.8	16.2	226	282	0.802	8.6	15.5	214	280	0.764

It seems probable that under the conditions of anoxemia the "lag" in oxygen intake at the beginning of a constant load of work can not be taken as an index of the "oxygen debt," or the amount of lactic acid that has accumulated in the body.

Observations during the recovery period. The influence of a reduced barometric pressure on the amount of oxygen consumed during physical exercise is clearly one of depression. By the amount that the intake of oxygen is reduced during work at the low pressure it would seem that the usage in

the period of recovery should be increased. However, such is not the case. Both at the normal and at the reduced barometric pressures there occurs an immediate rapid fall in the oxygen intake after work. The curves of the rate of return, examples of which appear in figure 3 and tables 4 and 5, are practically identical for the normal and low barometric pressure and fall one upon the other.

The total amount of oxygen consumed in the recovery period is usually approximately equal for any one subject at the several barometric pressures we have studied. Thus in R. W. C., in the first 22 minutes immediately after the exercise, the total intake of oxygen was 6149 cc. at 764 mm., 6141 cc. at 447 mm., and 6113 cc. at 415 mm. In a post period of 20 minutes H. M. used 6585 cc. at 760 mm. and 6543 cc. at 419 mm. In a series of 3 experiments on E. W. G. in 22 minutes after exercise, the consumption of oxygen was 7346 cc. at a barometric pressure of 756 mm., 7092 cc. at 457 mm., and 6827 cc. at 415 mm. In the 2 experiments on E. F. F. in 22 minutes the intake was 7968 cc. at 766 mm. and 7839 cc. at 457 mm.

The "oxygen debt," as ordinarily defined, is determined by measuring the total oxygen used in the selected period of recovery and subtracting from this the amount of oxygen which the body would have used in the same time during rest. In calculating this for our experiments at low barometric pressures we have been unable to decide whether the resting intake of oxygen at the normal or at the low barometric pressure should be used. In most of the experiments of this series the depressing effect of lowered barometric pressure on metabolism was in evidence when the metabolism was determined with the subject sitting at rest just prior to the work period. Since, in these cases of reduced metabolism, the intake of oxygen as found just before work began gives the highest values for the so-called oxygen debt we have used the low in preference to the normal barometric pressure determination in our statement of oxygen debt. The oxygen debt for R. W. C. according to the above measure was 1023 cc. at 764 mm., 1323 cc. at 447 mm., and 2131 cc. at 415 mm. If calculated on the basis of his resting metabolism at 760 mm. it would have been approximately the same for the 3 experiments. His metabolism during rest was more depressed at 415 mm. than in any other of our rather large number of observations on him, some of which were reported in another paper (Schneider, Truesdell and Clarke, 1924). From the reduction in the intake of oxygen during work at 447 mm. it was estimated that the oxygen debt would be increased 824 cc. above that present at sea-level pressure, making a total debt of 1847 cc. The oxygen debt as calculated above was 1323 cc., from which it appears that 524 cc. are unaccounted for. Likewise at 415 mm. an oxygen debt of 2606 cc. was expected and only 2131 cc. found, thus leaving 475 cc. unaccounted for.

In the experiments on H. M. an oxygen debt of 1445 cc. occurred at 760 mm. and only 843 cc. at 410 mm. The oxygen intake during work was reduced 2517 cc. at 410 mm. Therefore, the expected debt was 3962 cc., but instead it was less than at 760 mm. A similar result was obtained in the experiment with E. W. G. at 415 mm. His oxygen debt at 756 mm. had been 1010 cc., the decrease in oxygen consumption during work at 415 mm. led us to expect an increase of 1994 cc. or a total debt of 3004 cc. Instead it was found to be only 769 cc. In the experiment at 457 mm. an increase in the oxygen debt was found to be present, but it lacked 1226 cc. of being that expected. With E. F. F. the oxygen debt was found to be 2182 cc. at 766 mm. and 2119 cc. at 457 mm. The reduction in oxygen consumption during work at 457 mm. led us to expect an increase of 1193 cc. or a total debt of 3375 cc.

Had we used the sea-level rate of oxygen intake during rest as the basis for calculating, the oxygen debt would have been more nearly equal after a load of 4000 foot-pound for each subject in the several experiments conducted at the various barometric pressures. Even when the more favorable factor is used for the calculation, an increase in the oxygen debt is indicated for only two of the four subjects; and in not a single instance did the increase in debt anywhere near reach the theoretical amounts calculated as probable for work done at the low barometric pressures.

The initial phase of the process of recovery in the muscles of the body is rapid and is concerned with the oxidative removal of lactic acid from the muscles. Whatever acid escapes into the blood will require a much longer time to be oxidized or removed and this is a slow process that is revealed by a study of the second phase of recovery. Long and Lupton (1923) pointed out that immediately after exercise acid may be poured out of the muscles into the blood stream where it will obtain base from the bicarbonate and thus set free carbon dioxide. Hence in the earlier minutes after exercise, carbon dioxide should be excreted by the lungs in excess of that which is being produced by contemporary oxidation. It is during this part of the recovery that the respiratory quotient is greater than unity. There is good evidence that the use of oxygen in the muscles is such that the respiratory quotient for the oxidations during recovery is unity—lactic acid itself being oxidized. When later, in the second phase of the process of recovery, the body begins to oxidize the lactic acid that has escaped into the blood, some of the carbon dioxide formed, instead of being immediately eliminated by the lungs, will be used to combine with the base that is liberated by the oxidative removal of the acid. During this phase of recovery a considerable amount of carbon dioxide may be retained in the body. It becomes possible, therefore, to use the excess of carbon dioxide that is eliminated in the initial phase of the process of recovery as a rough measure of the amount of acid that has been formed in activity; and then again to use,

in the second phase, the retention of carbon dioxide as another rough measure of the lactic acid involved in activity. If during physical work at a low barometric pressure more lactic acid is formed in the muscles than during the same amount of work at sea-level pressure, then the period of excessive output, and later the period of retention, of carbon dioxide should give evidence of the presence of this larger amount of acid.

The carbon dioxide elimination of the post-exercise period of our experiments does not indicate that much more lactic acid was present in the body after exercise at low barometric pressures than at sea-level. Typical conditions are shown in tables 4 and 5. It will be observed that R. W. C. had a respiratory quotient above unity during the first 2 minutes after exercise at 764 mm., and not at all at 447 mm. One collection of expired air was lost at 415 mm., so our data are not complete enough in that experiment for a comparison. The retention of carbon dioxide during the second phase was 921 cc. at 764 mm. and 1254 cc. at 447 mm. H. M. had a respiratory quotient above unity for 2 minutes after work in both experiments, he eliminated a carbon dioxide excess of 228 cc. at 760 mm., but only 97 cc. at 410 mm. In the second phase he had a retention of 856 cc. at 760 mm. and 994 cc. at 410 mm. E. W. G. had a respiratory quotient above unity for 4 minutes after exercise in each of 3 experiments. The excess of carbon dioxide eliminated was 179 cc. at 756 mm., 479 cc. at 457 mm., and 215 cc. at 415 mm. The retention of carbon dioxide in the next 18 minutes reached 860 cc. at 756 mm., 598 cc. at 457 mm., and 733 cc. at 415 mm. The respiratory quotient of E. F. F. was above unity for 4 minutes at 766 mm. and for 7 minutes at 457 mm. The excess of carbon dioxide eliminated was 725 cc. at 766 mm. and 703 cc. at 457 mm. During the following 18 minutes the retention of carbon dioxide was 736 cc. at 766 mm. and 512 cc. at 457 mm.

The post-exercise elimination of carbon dioxide conforms with our observations on the "oxygen lag" during exercise and with those on the excessive consumption of oxygen after exercise (oxygen debt) in indicating that the decreased intake of oxygen during work under a low barometric pressure does not materially increase the amount of lactic acid in the body. Another indication that the reaction of the blood is not materially different after exercise at the low pressures is found in the minute volume of breathing. It will be observed in tables 4 and 5 that the breathing often returns to the pre-exercise volume within the 22 minutes following exercise. This, of course, would not occur if as much acid were discharged into the blood as is indicated by the reduced intake of oxygen.

SUMMARY

The subjective symptoms of anoxemia, such as light headedness and a disinclination to work, and some objective symptoms, such as cyanosis,

are ordinarily improved during work at a low barometric pressure. At barometric pressures corresponding to altitudes of from 15,000 to 25,000 feet the worker soon develops a marked feeling of air hunger and of muscular weakness. In no instance did work at a low pressure cause a headache.

Respiratory exchange determinations prior to the period of labor support the observation of a former study, to the effect that the oxidative processes of the body are retarded at barometric pressures less than 410 mm.

The interference of a low barometric pressure with the oxidative processes of the body is accentuated during physical work.

A linear relationship between the load of work and the amount of oxygen consumed per minute is maintained at all barometric pressures, up to that corresponding to an altitude of 25,000 feet, for normal loads of work. However, when a load is an over-load there is a reduction in the consumption of oxygen that causes a deviation in the line. This deviation is decidedly increased by additional lowering of the barometric pressure.

When the differences between the amount of oxygen consumed during a given load of work at the normal and at the low barometric pressures are plotted with respect to the barometric pressure the linear relationship is more clearly established.

The decrease in the oxidative processes of the body at a low barometric pressure is roughly proportionate to the increase in the size of the load of work.

In spite of the fact that the consumption of oxygen is markedly decreased during physical labor at a low barometric pressure, it is not shown that the oxygen debt of the body is increased by work at the low pressure. Theoretically the oxygen debt should be as much larger when work is done under a low pressure as the oxygen consumption at that pressure is less than when the same load is carried at the normal barometric pressure. Such is not the case. In the period of recovery after exercise the consumption of oxygen is approximately equal after equal loads of work at the normal and low barometric pressures. There is no indication in a post-exercise period of 22 minutes that the oxygen deficit is being made up at low barometric pressures.

Observations on the output of carbon dioxide after exercise at a low barometric pressure also indicate that the oxygen debt, or the degree of "acidosis," is not larger at a low than at the normal barometric pressure.

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PHENOLPHTHALOL, ITS PREPARATION AND REACTION TOWARD OXIDASES AND PEROXIDASES

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Refined methods have been developed which tell the sum total of the metabolic processes taking place in the animal body and to what extent there is an excess of catabolism or anabolism. These results or measurements leave untouched the many complex intermediate processes which occur before the end results are reached and of these intermediary reactions occurring in the animal body a comparatively small number are known.

There are oxidation reactions occurring in the animal body which are duplicated in vitro only by the aid of hydrogen peroxide. The similarity of reaction such as the production of glucuronic acid in the body and in vitro gives evidence which favors the existence of a peroxide or a superoxide in the living cells.

Kastle and Shedd (1901) demonstrated that phenolphthalin is an excellent reagent for the detection of oxidases and later Kastle and Amos (1906) showed that when hydrogen peroxide is added in small quantities to an alkaline solution of phenolphthalin, the oxidation is slow, but that the oxidation is greatly hastened by the addition of an oxygen carrier such as blood. Again, Kastle (1906) states that all of his attempts to oxidize phenolphthalin with extracts of animal tissues had failed and even when injected subcutaneously it passed apparently unchanged into the urine.

Kastle (1909) described the work done up to that time concerning phenolphthalin as a reagent for the detection of blood and showed that as little as one part in 8,000,000 could be detected by this reagent.

Kastle and Buckner (1917) have presented evidence of the possible

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existence of oxidation going on in the living cells of plants and that there is active oxygen in the protoplasm, since phenolphthalin was completely oxidized to phenolphthalein in the living cells of maize and okra.

Kastle (1906) showed that when phenolphthalein was injected intraperitoneally into a guinea pig it was passed out in the urine as a conjugated product which was detected by the addition of an alkali after hydrolysis with dilute hydrochloric acid and boiling.

In starting this investigation it was our desire to determine if phenolphthalol would be even more suitable as a reagent for the detection of blood than phenolphthalin and possibly give us some information concerning the oxidations of the body cells, and also to determine its use as a reagent in the detection of oxidases and peroxidases.

In the year 1912, Dr. Joseph H. Kastle told the author of this paper that he and several others working in his laboratory had attempted to prepare an organic compound called "phthalol" (phenolphthalol, $C_{20}H_{18}O_3$) that had been prepared and described by Adolf Baeyer (1880). It is originally described as the product obtained by reducing phenolphthalin ($C_{20}H_{16}O_4$) by sodium-amalgam in acetic acid solution. Doctor Kastle stated that the description of the reduction of phenolphthalin to phenolphthalol was such that, when followed, the final product was always phenolphthalin with a melting point of 225°C. instead of 190°C. which is the melting point of phenolphthalol. At that time an attempt was made by the author to prepare this compound and after many unsuccessful efforts it was abandoned for the time.

In 1923 the author, working in the biochemical laboratories of the Pasteur Institute, Paris, France, attempted to prepare phenolphthalol, with the ultimate view of studying its reactions toward oxidases and peroxidases and to determine if it was possible to use it as a means of detecting oxidations going on in the animal body.

Preparation of phenolphthalol. Phenolphthalin which gave no test for phenolphthalein was prepared according to the directions of Kastle (1909). After following closely the directions of Baeyer (1880) for preparing phenolphthalol from phenolphthalin with no success, the temperature, concentrations of reagents and the speed and durations of the reactions were varied and it was finally prepared according to the following directions:

Two grams of pure, freshly prepared phenolphthalin were placed in 400 cc. of distilled water in an 800 cc. beaker and just enough 50 per cent acetic acid was added through a dropping funnel to cause the phenolphthalin to go into solution while the solution was actively boiling. The boiling was continued and the solution kept at a constant volume by the addition of distilled water and during 3 hours 500 grams of 4 per cent sodium amalgam were added in small quantities so that the reduction

was continuous. More acid was added from time to time in quantity sufficient to keep the solution slightly acid during the reduction. The boiling solution was diluted with distilled water until the solution became milky. It was then allowed to cool and stand over night. The crystalline precipitate which appeared was filtered out and washed thoroughly with cold distilled water. It was then dissolved on the filter with the smallest quantity of boiling glacial acetic acid. The hot filtrate was diluted with distilled water until it became milky. It was then heated to boiling, which caused the solution to become clear, and afterwards allowed to crystallize out. After this it was filtered and washed with cold distilled water and dried at 40°C. These crystals were faintly straw colored, being prismatic in shape and having a melting point of 190°C.

Reaction toward oxidases and peroxidases. A solution was made by putting 0.01 gram of phenolphthalol into 20 cc. of distilled water and adding enough alcohol to dissolve it. This solution gives no color with NaOH, but the alkaline liquid becomes deep pink on adding a little K_3FeCN_6. When the solution of phenolphthalol is allowed to stand exposed to the air for some time, however, it gives a red color on adding NaOH, showing oxidation; for this reason, only freshly prepared solutions were used in all the following experiments.

To test its activity toward oxidases, the fresh solution of phenolphthalol, prepared as described above, was added to a solution made by mixing 2 grams of scrapings from the skin of a raw potato with enough water to make 100 cc. and filtering. The following comparisons were made:

1. 5 cc. distilled water + 2 cc. of phenolphthalol solution.
2. 5 cc. of extract of potato skin + 2 cc. of phenolphthalol solution.
3. 5 cc. of extract of potato skin boiled + 2 cc. of phenolphthalol solution.

These solutions after standing 15 minutes were made alkaline with N/10 NaOH and gave the following reactions:

No. 1. No color.

No. 2. Deep pink.

No. 3. Very pale pink.

This showed the presence of an oxidase. The result was identical with that given by phenolphthalin.

In testing for peroxidases in soybeans, phenolphthalol was found to react in every way as did phenolphthalin and to the same extent.

Blood may be detected accurately in solutions of 1 part in 5,000,000 but in greater dilutions it is not reliable. Kastle was able to detect blood in dilutions of 1 to 8,000,000 by using phenolphthalin.

In order to determine the fate of phenolphthalol in the animal body, 0.016 gram of the pure substance was dissolved in 5 cc. of N/10 NaOH, a solution of hydrogen peroxide containing 0.034 gram of H_2O_2 was added

and the whole made to 25 cc. with distilled water. Five cubic centimeters of this solution were injected intraperitoneally into a male guinea pig weighing 570 grams. At the same time, 0.05 gram of powdered phenolphthalol was suspended in 5 cc. of distilled water and injected intraperitoneally into another male guinea pig weighing 607 grams. No phenolphthalol was excreted in the urine of either pig, during the first 40 hours following the injection. After this lapse of time it was excreted continuously in small quantities in the urine for 14 days. There was no evidence of any oxidation nor was any conjugated product formed.

Fresh urine does not oxidize phenolphthalol but urine that has been voided for 10 hours or longer will oxidize phenolphthalol to phenolphthalein; this is the result of bacterial action. However, the oxidation was never complete under these conditions, as was shown by further oxidation with K_3FeCN_6.

In other words, phenolphthalol is not oxidized to phenolphthalein in the body of the guinea pig. These experiments have been duplicated and always with the same results. The guinea pigs suffered no discomfort from the injections nor was diarrhea caused in any case.

With the view of studying the conduct of phenolphthalol in a cold-blooded animal which was devoid of hemoglobin, the following tests were made at the Oceanographic Institute at Monaco, using the mollusc *Tapes decussatus*, which was obtained from the Mediterranean Sea near Marsailles, France.

The fresh Mediterranean sea water obtained at Monaco gave no color with phenolphthalein and did not oxidize phenolphthalin or phenolphthalol, with or without the addition of hydrogen peroxide, thereby showing that it contained neither an oxidase nor a peroxidase. This knowledge was essential since the reactions with the live mollusc must be made in the sea water.

Two molluscs of the species named were placed in separate dishes, each containing 200 cc. of fresh sea water. One cubic centimeter of phenolphthalin solution was added to one, and 1 cc. of a solution of phenolphthalol to the other. After 12 hours the water in each was made alkaline with sodium hydroxide which caused no development of a pink color in either case. However, a deep pink was developed by the addition of K_3FeCN_6.

Neither the excrement nor the blood of *Tapes decussatus* gave the oxidase or the peroxidase tests with phenolphthalin or phenolphthalol.

The body of a *Tapes decussatus* was dissected from the shells and dried in the air at room temperature; it turned brown in color and became horny in nature. This dried material when ground in distilled water formed a milky suspension. This solution gave no test for oxidases with either phenolphthalin or phenolphthalol. However, it did give a strong peroxidase reaction with both reagents when H_2O_2 was added.

A *Tapes decussatus* was dissected into the following parts and dried:

1. Two syphons.
2. Foot.
3. Organs of the abdomen and their contents.
4. Outer mantles dissected from edge of shells.

None of these parts gave an oxidase test with phenolphthalin or phenolphthalol and only in the case of no. 3, the abdominal organs and their contents, was a positive peroxidase test obtained and this was strong with both reagents.

A small hole was cut in one of the halves of the shell of a *Tapes decussatus* exposing the abdomen. Into the abdomen was injected 0.25 cc. of a solution of phenolphthalin and into another a solution of phenolphthalol. The molluscs were then placed separately in sea water and after 24 hours the water was tested; no phenolphthalein was indicated when the water was made alkaline. The bodies of these molluscs were then dissected out and ground in a mortar to a paste. This material showed no evidence of containing an oxidase or a peroxidase.

CONCLUSIONS

A detailed method is described whereby phenolphthalol can be made easily.

From the foregoing experiments it seems that phenolphthalol is an excellent reagent for the use in detecting oxidases and peroxidases but that, owing to the added trouble in making it, phenolphthalin is preferable.

Phenolphthalol, when injected into the abdomen of a guinea pig or a mollusc such as *Tapes decussatus*, is passed out unchanged, showing that it is not oxidized by the living cells of these two animals.

The fresh organs of *Tapes decussatus* do not give the oxidase or peroxidase tests with phenolphthalin or phenolphthalol; nor does the dried material give an oxidase or peroxidase test with these reagents except when a positive peroxidase reaction is given with the dried organs of the abdomen and their contents.

The blood of the *Tapes decussatus* and the excrement do not contain an oxidase or peroxidase which can be detected by phenolphthalin or phenolphthalol.

Human blood can be detected with certainty in dilution of 1 part in 5,000,000 by the peroxidase test with phenolphthalol.

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EGG WHITE VS. CASEIN AS SOURCE OF PROTEIN IN THE DIET OF RATS

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Some years ago in searching to find a natural source of protein convenient for laboratory purposes, which would be comparatively low in calcium and phosphorus, the use of egg white was adopted. Osborne and Mendel (1) had found 18 per cent of egg white adequate for normal growth if the other factors in the diet were supplied. Bateman (2) also used egg white successfully but in reviewing the literature discovered reports of failures which were undoubtedly due to lack of some of the vitamins. More recent work by Boas (3) concludes that egg white is inadequate as the sole source of nitrogen for young growing rats even when it amounts to 20 per cent of the total ration and other necessary factors are supplied.

In the light of our recent findings we cannot agree with Boas (3) that egg white is an extremely inadequate source of protein but a more precise comparison of the relative values of egg white and casein may be of interest to other research workers. Our first observations were made on standard 18 per cent protein diets in which egg white had been substituted for casein for convenience in controlling the mineral content of the food. Later a more extensive comparison of the two proteins at different levels was undertaken.

Our observations have been made on the following food mixtures with results as indicated in the accompanying figures.

	I	II	III	IV	V	VI
Egg white	19	18	19	5.6	11.2	16.8
Cornstarch	50	42	67	78.4	72.8	67.2
Lactose		15				
Agar				2	2	2
Salt mixture	4	4	4	4	4	4
Crisco	18	12				
Butter fat	9	9	10	10	10	10

Hard boiled egg whites were prepared by thoroughly washing in distilled water, grinding and drying at a temperature of 80 or 90°C. The yellowish

crystal-like product resulting was then ground to a consistency of granulated sugar and was used in that form in making up all foods. The dried

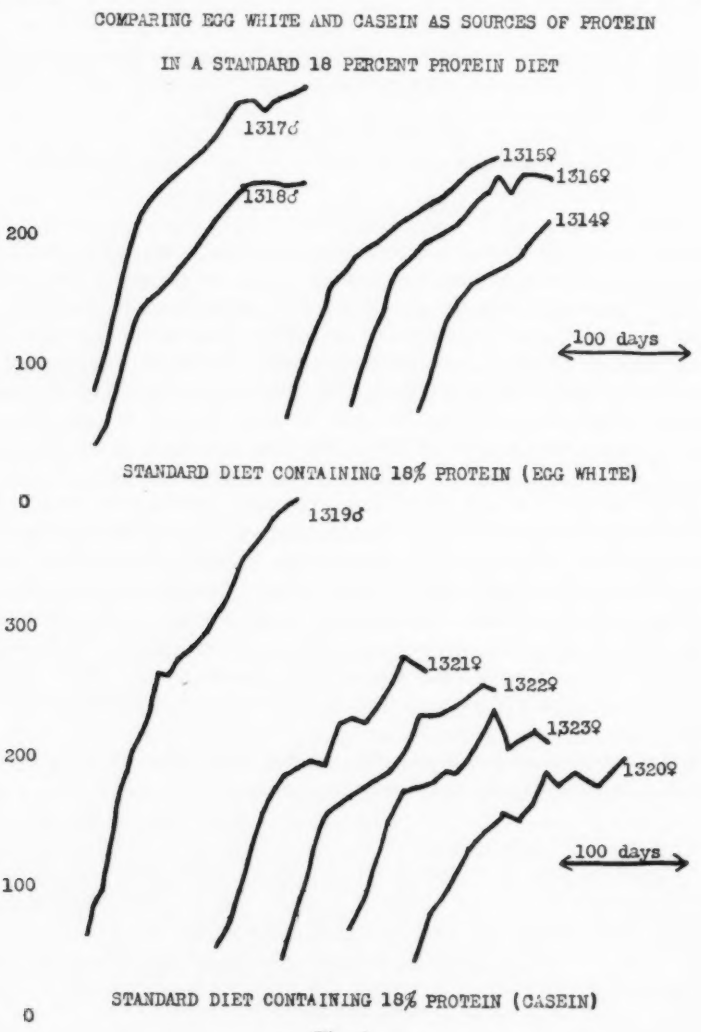

Fig. 1

egg white contains about 89 per cent of protein, and only 0.08 per cent of calcium and 0.084 per cent of phosphorus, and small traces of other food constituents.

Foods I and II were made up as paste foods according to Osborne and Mendel; all others were baked in the form of biscuits. We have found the latter form more satisfactory as the animals like it better, scatter it less and there is no tendency for the formation of hair balls in the stomachs, which occurred frequently on paste foods (4).

Wherever egg white had been used to furnish 18 per cent or more of

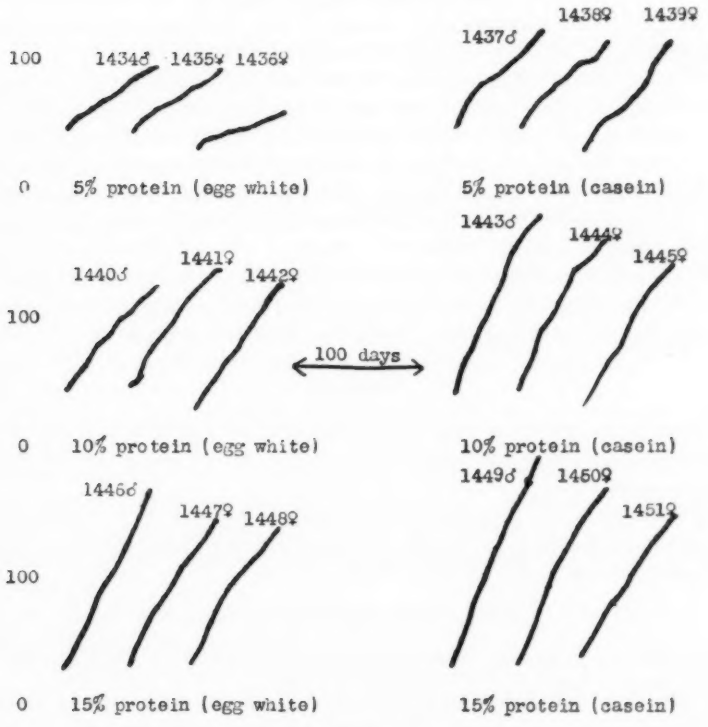

Fig. 2.

protein in the diet and yeast was the source of vitamin B, perfectly normal growth had always been attained although reproduction on such diets was somewhat poorer than where casein was the source of protein. However, since yeast furnished a significant amount of supplementary nitrogen further experiments were conducted to compare the relative value of casein and egg white using a yeast concentrate (Harris) to avoid supplying

any trace of supplementary protein or amino acid. The results of this test are shown in figure 1, which indicates that the casein holds a slight advantage over the egg white in the more rapidly growing males but the females show approximately equal growth.

In order to still more accurately compare the relative value of these proteins, rats have been fed on diets containing 5, 10 and 15 per cent respectively of protein from each of these sources. Harris yeast concentrate (protein free) again supplied the vitamin B. The results are indicated in figure 2. Casein is consistently just slightly better than the egg white but the higher the percentage used the less pronounced the difference. This suggests that enough of the limiting factor is probably supplied in the higher percentages to make good the deficiency.

The suggestion made by Boas (3) that the egg white may be lacking in cystine and thus account for the loss of hair observed in their rats is not applicable in our findings. In no case have we observed loss of hair in rats on diets in which egg white furnished anywhere from 5 to 50 per cent protein. The rats always appeared in good condition with sleek coats, the only evidence of malnutrition being the stunted growth on the lower percentages of protein.

For practical purposes where dry yeast supplies the vitamin B, egg white is a very satisfactory source of protein and is especially adapted for the preparation of rachitic diets (5) or for any diets low in minerals.

SUMMARY

The relative values of egg white and casein have been determined showing that egg white is just slightly less adequate than casein for the growth of young rats.

When egg white furnishes 18 per cent of protein in a standard diet supplemented with yeast it is an adequate source of protein and especially adapted for use in low mineral diets.

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THE HYDROGEN ION CONCENTRATIONS AND BASICITY OF EGG YOLK AND EGG WHITE

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Wishing to add fresh egg yolk to a medium used in an effort to cultivate the causative agent of hog cholera, and failing to find in the literature a record of the C_H of fresh egg yolk, one of us (Healy) determined the C_H of egg yolk and egg white colorimetrically. The marked yellow color of the egg yolk presenting somewhat of a difficulty, the following method was used: A drop of fresh egg yolk, and a drop of indicator were placed side by side on a clean white porcelain slab and carefully mixed, using a platinum needle for this purpose. Bromthymol blue and bromcresol purple gave the best results, and of these bromcresol purple gave the most satisfactory reading. The indicators were used in 0.02 per cent solution in water, to which was added sufficient N/20 NaOH to form the sodium salt.[1] The determinations noted in table 1 were made within a few hours after the eggs were laid.

Having separated the whites from the yolks for the purpose of the above determinations it seemed natural to determine the C_H of the whites. In testing the egg white, thymol blue gave the most satisfactory reading, and was used in a 0.02 per cent solution in water with sufficient N/20 NaOH added to give the sodium salt. Using the above drop method the determinations noted in table 2 were made on the whites of the eggs noted in table 1.

As there is but a very delicate membrane between the yolk and white these results were surprising.

To determine what changes might occur in the C_H of the whites and yolks of eggs with the lapse of time, two dozen strictly new-laid eggs were placed on the laboratory table. Three of them were examined at once and the remainder at stated periods with the results shown in table 3.

On the eighth day the egg white was nesslerized yet no ammonia was detected. It is interesting to note that while the C_H of the egg white rapidly changed, the C_H of the egg yolk remained practically constant for three weeks.

[1] Clark, W. M. The determination of hydrogen ions. Baltimore, 1920.

To determine the effect of incubation on the C_H of eggs, two dozen new-laid, supposedly fertile eggs, were placed in a Lectro-Hatch incubator at

TABLE 1

EGG	BROMCRESOL PURPLE	BROMTHYMOL BLUE
Yolk 1	pH 6.2	pH 5+, or 6
Yolk 2	pH 6.6	pH 5+, or 6
Yolk 3	pH 6.4	pH 5+, or 6

TABLE 2

EGG	THYMOL BLUE
White 1	pH 8.2
White 2	pH 8.2
White 3	pH 8.2

TABLE 3

TIME INTERVAL	EGG YOLK	EGG WHITE
New laid eggs	pH 6.2	pH 8.2
	pH 6.6	pH 8.2
	pH 6.4	pH 8.4
48 hours	pH 6.3	pH 9.0
	pH 6.3	pH 9.0
	pH 6.3	pH 9.1
96 hours	pH 6.4	pH 9.3
	pH 6.4	pH 9.4
	pH 6.4	pH 9.5
8 days	pH 6.2	pH 9.5
	pH 6.3	pH 9.5
	pH 6.2	pH 9.5
14 days	pH 6.4	pH 9.6
	pH 6.4	pH 9.6
	pH 6.4	pH 9.6
21 days	pH 6.3	pH 9.6
	pH 6.3	pH 9.6
28 days	pH 6.6	pH 9.7
56 days	pH 7.2	pH 9.4

100° to 103°F., and to determine the effect of an atmosphere of CO_2 one dozen new-laid, infertile eggs were placed in a Novy jar and CO_2 passed

through the jar for 30 minutes, the jar was then closed and placed on the laboratory table. One dozen new-laid, infertile eggs placed on the laboratory table served as checks. C_H determinations were made at the beginning of the experiment and at stated intervals thereafter with the results shown in table 4. After opening the Novy jar to take out eggs, more CO_2 was passed through it.

On opening the Novy jar on the forty-first day there was a distinct odor of H_2S, the interior of the jar and the exterior of the eggs were moist, and there was a growth of mold on two eggs.

TABLE 4

CONDITION	NEW-LAID EGGS		AFTER 3 DAYS		AFTER 6 DAYS		AFTER 13 DAYS		AFTER 41 DAYS	
	Yolk	White	Yolk	White	Yolk	White	Yolk	White	Yolk	White
	pH	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*
Fertile	6.3	8.2	6.8	9.4	6.8	8.2				
Infertile	6.3	8.2	6.4	9.2	6.3	9.4	6.6	9.7	6.6	9.0
In CO_2			6.3	7.8	6.3	7.6	6.4	7.2	6.3	6.4

TABLE 5

pH and neutralizing power of eggs kept at room temperature. Ten grams titrated after lapse of times stated

TIME INTERVAL	YOLK		WHITE		
	pH	0.1n NaOH phenolphthalein	pH	0.1n HCl phenolphthalein	0.1n HCl methylorange
		cc.		*cc.*	*cc.*
New-laid	6.4	6.6	8.3	0.3	15.2
48 hours	6.3	6.8	9.0	1.2	14.4
96 hours	6.4	6.9	9.4	1.4	13.3
8 days	6.2	7.0	9.5	1.5	13.3
14 days	6.4	6.0	9.6	1.6	13.3
21 days	6.3		9.6	1.7	14.8
28 days	6.6	4.9	9.7	1.7	15.5
56 days	7.2	5.7	9.4	1.6	15.5

Table 4 shows that incubation at first diminishes the C_H of both the egg yolk and egg white but that later, with the development of the chick and with the production of CO_2, the C_H of the egg white increases.

Table 4 further shows that an atmosphere of CO_2 markedly increases the C_H of the egg white, while the C_H of the egg yolk remains practically constant.

After the C_H had been determined, 10-gram portions of white and yolk from the eggs used for the C_H determinations were mixed with 40 cc. of distilled water and titrated; the yolks with 0.1n NaOH, using phenolphthalein, and the whites with 0.1n HCl, using, first, phenolphthalein and

then methyl orange. The number of cubic centimeters of 0.1n HCl stated in the last column as the titration with methylorange, in each case, is the total quantity used, including the number of cubic centimeters stated in the next to the last column as the titration with phenolphthalein.

TABLE 6

pH and neutralizing power of infertile eggs kept at room temperature. Ten grams titrated after lapse of times stated

TIME INTERVAL	YOLK		WHITE		
	pH	0.1n NaOH phenolphthalein	pH	0.1n HCl phenolphthalein	0.1n HCl methylorange
		cc.		cc.	cc.
72 hours	6.4	6.5	9.2	1.3	13.5
6 days	6.3	5.9	9.4	1.3	13.5
13 days	6.6	5.7	9.7	2.0	13.5
41 days	6.6	5.3	9.0	0.9	15.5

TABLE 7

pH and neutralizing value of infertile eggs kept in CO_2 at room temperature. Ten grams titrated after lapse of times stated

TIME INTERVAL	YOLK		WHITE		
	pH	0.1n NaOH phenolphthalein	pH	0.1n HCl phenolphthalein	0.1n HCl methylorange
		cc.		cc.	cc.
72 hours	6.3	7.0	7.8	0.	12.0
6 days	6.3	8.9	7.6	0.	14.5
13 days	6.4	7.2	7.2	(0.5)*	15.5
41 days	6.3	11.3	6.4	(1.5)*	14.5

*0.1n NaOH. Shows presence of free CO_2

TABLE 8

pH and neutralizing value of fertile eggs, incubated. Ten gram portions titrated after lapse of times stated

TIME INTERVAL	YOLK		WHITE		
	pH	0.1n NaOH phenolphthalein	pH	0.1n HCl phenolphthalein	0.1n HCl methylorange
		cc.		cc.	cc.
72 hours	6.8	5.2	9.4	1.4	15.3
6 days	6.8	12.9	8.2	1.7	14.8

The findings are given in tables 5 to 8, together with the corresponding pH findings taken from tables 3 and 4. Averages of two or three findings, both of pH and of titrations, are used in table 5 wherever table 3 indicates that two or three eggs of the same age were tested. Endpoints in the

titrations were not satisfactory, and the exposure of the material to the air during the varying intervals between the measurement of pH and the titration must have caused irregularities from escape of CO_2.

Presumably, the greater part of the basicity shown by the titration with 0.1n HCl and methylorange is due to carbonate. It is affected, however, by the small amount of phosphte present, and possibly by other reacting substances.

Independent tests, applied immediately after opening, to the white of eggs known to have been laid not more than an hour or two previously, showed no color with phenolphthalein. On standing in an open beaker, however, the surface of the egg white exposed to the air became pinkish after a few minutes, but the color was destroyed by stirring the mass. On longer standing, however, the color became permanent and increased with lapse of time. This behavior resembles that of a water solution of acid sodium carbonate ($NaHCO_3$) containing excess of CO_2. The experiment was tried of adding phenolphthalein to such a solution and exposing it to the air, at room temperature, in an open beaker. Originally colorless, the solution became pinkish within 15 or 20 minutes, and the color darkened gradually with lapse of time. The C_H of a weak solution of commercial $NaHCO_3$ was found to be pH 8.6, measured colorimetrically; that of a similar solution through which CO_2 had been bubbled was pH 7.8, measured with cresol purple. On exposure to the air this solution rapidly became more alkaline and it was apparent that pH 8.2, observed in fresh egg white, could be obtained easily with such a solution. Distilled water through which CO_2 had been bubbled, measured pH 5.2 with methyl red.

The presence of sodium and potassium acid carbonates in egg white is consistent with published analyses of the ash, which show an alkaline condition, with sodium and potassium oxids comprising the largest part of the bases.

The reaction of egg white to phenolphthalein affords a means of proving the freshness of eggs which have been kept under normal conditions; that is, not specially treated. If the white of an egg, tested immediately after breaking, gives no color with phenolphthalein, the inference is that the egg was laid within a very few hours. The depth of color increases with age for the first few days.

SUMMARY

The H-ion concentrations of egg yolk and egg white were measured colorimetrically.

The average C_H of five measurements of the yolks of new-laid eggs was pH 6.36, while the average C_H of the measurements of the whites of the same eggs was pH 8.24.

The C_H of egg white rapidly changed with the lapse of time, whereas the C_H of the yolk remained practically constant for three weeks.

During the first few days of incubation the C_H of both egg white and yolk diminishes and, with the development of the chick, the C_H of the egg white increases.

In an atmosphere of CO_2 the C_H of the egg white distinctly increases, whereas the C_H of the yolk remains practically constant.

The behavior of egg white to indicators suggests the presence in it of sodium or potassium acid carbonate, with excess of carbon dioxid.

The reaction of egg white to phenolphthalein is suggested (Peter) as a test of freshness.

IS PROLONGED BED REST A PREREQUISITE FOR THE MEASUREMENT OF BASAL METABOLISM?

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The term "basal metabolism" as currently accepted, at least in America, implies the minimum of energy necessary to keep the human machine functioning normally while in repose, awake, and 12 hours after the last meal. Of the various factors known to influence metabolism, two at least (the influence of muscular work and the after-effect of taking food) are ruled out by the conditions ordinarily imposed, for only those experiments that are made with the subject in the post-absorptive condition and after 30 minutes of complete muscular repose are commonly considered as meeting the requirements for basal metabolism. Other factors, such as sleepiness, exposure to a cold environmental temperature and psychic disturbances, are tacitly recognized and assigned a significance depending upon the critic's actual experimental experience (not infrequently lack of experience) with the subject. Even the factors of the post-absorptive condition and muscular repose are not fixed with rigidity, for the post-absorptive condition (12 hours after the last meal) presupposes that the last meal has not been excessively rich in protein, and the rest period of 30 minutes prior to the experiment has been challenged as being unnecessarily long.

It is universally conceded that for basal metabolism measurements subjects must be lying, well covered and comfortable. As a matter of fact, in most of the Nutrition Laboratory work the great difficulty has been to keep subjects awake. This of itself speaks for the great comfort which must obtain under Nutrition Laboratory conditions.

The inflexibility and the lability of basal metabolism are treated almost in the same breath by various writers. In general the variations in the basal metabolism of an individual upon whom measurements have been made repeatedly over many years are by no means so wide as the variations between different individuals, and yet it cannot be said that each person has a fixed basal metabolism that does not change. The elements of digestion and muscular activity are supposedly ruled out by the prescribed conditions of the experiment. Are there other important factors for which allowance should be made?

The recent criticisms of Lefèvre (1922) with regard to the supposedly rather appreciable influence of environmental temperature have been experimentally studied in the Nutrition Laboratory (Benedict and Benedict, 1924) as well as by Delcourt-Bernard and Mayer (1925) in Paris, and apparently submersion in a neutral bath at about 35°C. does not lessen heat production, whatever its effect upon heat loss may be. Of special interest is the suggestive finding of Mayer in a few of his numerous experiments that after the bath, when the subject is again dressed and lying on the bed, covered, there is an "after-effect" of the bath in the direction of a lower metabolism.

Recently it has been suggested that the minimum metabolism may not be secured until after a considerable period of rest in bed (Benedict, 1924). Metabolism studies of girls showed that the Nutrition Laboratory series of Girl Scouts and the girls studied by Benedict and Talbot had lower metabolism figures than groups studied in Chicago and New York in two other laboratories (Benedict, 1924). This suggested that the element of rising, dressing, walking, exposure to outdoor air and mounting stairs, which was present in the case of the New York and Chicago groups and was absent in the Nutrition Laboratory group, might account for the generally lower metabolism noted with the latter group. The conditions which ordinarily surround the determination of basal metabolism in the physician's office and not infrequently in the hospital clinic involve a journey from the patient's home after rising and dressing in the morning. He then lies down for the prescribed half hour. These conditions are more nearly like those which obtained in the measurements of the New York and Chicago groups of girls, while the conditions for those patients who remain in the hospital over night and have their metabolism measured in the morning are more in conformity with conditions obtaining in the majority of the Nutrition Laboratory experiments on girls.

Is the metabolism measured in the morning before the subject gets out of bed, with the body thoroughly warm and relaxed and in an even environmental temperature, lower than that measured an hour later after the subject rises, perhaps bathes, dresses, and walks under various atmospheric conditions to the physician's office or the laboratory, even if the muscular movement incidental thereto is followed by a half-hour of repose? At the present time it is generally believed that the metabolism after a night's rest in bed may be lower and hence nearer the true basal than under the other prevailing conditions. It is obviously a matter of great practical importance whether it is necessary that a much used and frequently much needed hospital bed be sacrificed for a night to insure basal conditions. To put this question to a test, a number of experiments were made on one of us in the winter of 1924, which indicated that

the difference between the two conditions was very much less than one would suppose. Hence we planned a series of well-controlled experiments with a homogeneous group of young college women, thoroughly coöperative and living under easily controlled conditions as to diet, temperature of sleeping room and activity after rising.

Two features in the measurement of the basal metabolism in hospitals or clinics to which patients greatly object are the fact that they should spend the night at the hospital and that they should be without breakfast. The second of these objectionable features has been studied in the Nutrition Laboratory and a satisfying, non-stimulating breakfast has been proposed (Benedict and Benedict, 1923a). This present investigation throws light upon the question as to whether a sojourn in the hospital or a night of bed rest is necessary prior to basal metabolism measurements.

General plan of research. The general plan of the research called for the determination of the basal metabolism after the subject had rested quietly throughout the night in a comfortable bed, well covered and under conditions favorable for a good night's sleep. After the subject woke in the morning and while the body was still relaxed and warm and the skin temperature was reasonably uniform, two metabolism measurements were made. The subject then rose and went through the usual morning toilet, including in many instances a shower bath, dressed as usual, walked down three flights of stairs and then walked out of doors for 10 minutes, returned to the laboratory, walked up the three flights of stairs, and lay down for one-half hour in the laboratory room, lightly covered with a blanket. The metabolism was then again determined in two periods.

Following a few preliminary experiments made upon one of us, an extended study was made with a group of young college women, whose habits of life were very regular, and who were all considered to be physically in good health. Two rooms on the fourth floor in the new Cornelia Clapp Biological Laboratory of Mount Holyoke College, at South Hadley, Massachusetts (fitted with every convenience for comfortable overnight sojourn and toilet and bathing facilities) were set aside for this work. In one room, a large laboratory, two subjects slept in two beds, well covered, and in the adjoining room two trained operators slept. In the morning the operators rose, closed the windows in the laboratory, and brought the respiration apparatus in from the hall, where it had been placed to avoid extremes in temperature. Two respiration experiments were then made immediately on each subject. The respiration apparatus used was a valve modification of the Benedict and Collins apparatus (Benedict and Benedict, 1923b). Mouth temperatures were taken, chiefly to demonstrate the absence of a febrile condition. The pulse rate was recorded during the determinations and a survey of the skin temperature by the thermo-electric method (Benedict, Miles and Johnson. 1919) was frequently made.

After this series of measurements, the young women dressed and went through the routine outlined above. After their return to the laboratory and after one-half hour's repose, two more respiration experiments were made and the session ended. Observations were made upon 7 young women on from 3 to 9 different days in each case. These young women ranged in age from 18 to 23 years.

DISCUSSION OF RESULTS. While it was the original plan of the research to deal solely with the measured metabolism and in the preliminary series of experiments on subject I only the oxygen consumption was measured, in the expanded series data were also collected on skin temperature and pulse rate. The preliminary experiments will first be considered.

Experiments with subject I. The first observations on this general theme were made on one of the writers (F. G. B.) on February 26, 1924. A well-tested student form of respiration apparatus (Benedict and Benedict, 1923b) was taken to the house and placed at the head of the bed. In the morning, before subject I rose, two respiration experiments were made by a skilled operator (Mrs. Benedict). Since the length of time required for the absorption of a definite amount of oxygen, as introduced by the meter pump, showed good agreement in these two experiments, a third measurement was not made. The subject then rose, dressed, and went to the Laboratory, walking about 20 minutes. The walking was poor, as there was considerable ice and the footing was rather bad. After entering the Laboratory, the subject climbed three flights of stairs and lay down upon a cot in one of the Laboratory rooms. Following the usual half hour of rest, three experimental periods were made by Mr. E. L. Fox with a Benedict and Collins (1920) respiration apparatus. As usual the graphic records on the kymograph were obtained and the test for tightness was made by placing a weight upon the spirometer bell in the middle of the experiment.

On February 28 the experiment was repeated exactly as on February 26. Since much of the snow and ice had melted, the walking was a little better. Two periods were made prior to the subject's leaving the house and three periods after he arrived at the Laboratory. On February 29 and on March 1 the series was again repeated. The outdoor temperature was 0°C. on February 29 and −3°C. on March 1. The outdoor temperature was unfortunately not recorded on February 26 and 28.

In discussing these comparison experiments one is confronted by the fact that, theoretically, exactly the same apparatus should have been used for both series. As a matter of fact, repeated tests, both alcohol check tests and tests with humans, had shown that the two forms of apparatus gave identical results with the same person.

The study with subject I centers around the measurement of the oxy-

gen consumption per minute, the data for which are given in table 1. Usually the two measurements at the house agreed well, although on February 28 and March 1 there was a difference of 12 cc. in each case. In the experiments at the Laboratory there is in no case an indication of an increased metabolism following the exercise of rising, dressing, walking approximately 20 minutes, and climbing three flights of stairs. Indeed there is a tendency for the results obtained at the Laboratory to be slightly lower, on the average, than those obtained at the house. It may be a mere coincidence that the lowest figure, 239 cc., was found during one of the periods at the Laboratory.

This series of experiments, which must be looked upon solely as preliminary, justifies the general conclusion, however, that the activity incidental to rising in the morning (as outlined above) has no measurable influence upon the basal metabolism, as indicated by the oxygen con-

TABLE 1

Oxygen consumption (cubic centimeters per minute) before and after rising in the morning
Subject I

DATE	OUTDOOR TEMPERATURE	LYING, BEFORE RISING*		LYING 30 MINUTES, AFTER RISING, DRESSING, AND WALKING 20 MINUTES†		
		Period 1	Period 2	Period 1	Period 2	Period 3
1924	°C.					
February 26		251	249	249	249	242
February 28		252	240	241	241	239
February 29	0	254	250	256	255	253
March 1	−3	259	247	257	255	244

* The student form of respiration apparatus was used in these periods.
† The Benedict-Collins respiration apparatus was used in these periods.

sumption. The importance of this observation from a practical standpoint, in connection with the technical routine of determining basal metabolism in hospitals, particularly in follow-up cases where repeated measurements must be made, justified a further study of the problem, which was carried out with the group of young college women at Mount Holyoke College, South Hadley, Massachusetts.

Experiments on a group of college women. To observe what might be the influence upon basal metabolism of such activity following bed rest at night in the case of a group of untrained young women, a series of experiments was planned for the winter of 1924–1925. Realizing that there would undoubtedly be rather extensive changes in peripheral temperature, we took advantage of the opportunity to measure the skin temperatures of the subjects just prior to rising and after they had returned from their walk in the college grounds. In this study our prime object was

TABLE 2

Oxygen consumption, pulse rate, and skin temperature of college women before and after rising

(Average values)

SUBJECT AND DATE	OUTDOOR TEMPERATURE	OXYGEN CONSUMPTION PER MINUTE		PULSE RATE		SKIN TEMPERATURE		
		Before rising	After rising	Before rising	After rising	Before rising	After walk	Lying covered
1924-1925	°C.	cc.	cc.			°C.	°C.	°C.
Subject II:								
October 22	4.4	268	261	56	49			
October 27	3.3	241	255		47			
October 29	7.2	235	235	55	50			
October 31	−3.3	234	237	60	54			
November 10	0.0	239	248	54	51			
November 12	8.3	247	246	58	53			
February 4	−8.3	247	268	56	52	31.8	27.3	29.6
February 6	0.0	236	242	53	52			
Average		243	249	56	51	31.8	27.3	29.6
Subject III:								
October 23	−2.8	208	205	54	57			
October 28	6.1	195	201	68	65	31.4	27.7	31.2
October 30	2.2	188	197	62	64	31.4	27.3	29.2
November 1	4.4	195	189	64	62			
November 11	−2.8	190	203	66	65	32.4	26.3	29.3
November 13		189	197	65	64			
February 3	−10.6	195	199	64	66	32.3	26.3	
February 5	−11.1	180	195	71	62	31.6	27.5	29.8
February 7	0.0	193	181	69	62	31.1	26.9	29.8
Average		193	196	65	63	31.7	27.0	29.9
Subject IV:								
October 24	2.2	194	202	54	53			
October 27	3.3	192	204	52	53			
October 29	7.2	196	201	52	53			
October 31	−3.3	185	193	55	56			
February 11	3.3	184	198	60	56			
February 13	−5.6	191	203	61	56			
Average		190	200	56	55			
Subject V:								
October 28	6.1	172	175	57	58	32.5	28.6	31.4
October 30	2.2	161	170	57	55	32.0	28.2	29.6
November 1	4.4	175	176	61	54			
February 10	2.2	177	177	53	52	31.1	29.8	31.6
February 12	5.6	173		58	60	33.2	30.0	32.5
February 14	−3.3	179	176	56	55	31.7	25.5	28.7
Average		173	175	57	56	32.1	28.4	30.8

TABLE 2—*Concluded*

SUBJECT AND DATE	OUTDOOR TEMPERATURE	OXYGEN CONSUMPTION PER MINUTE		PULSE RATE		SKIN TEMPERATURE		
		Before rising	After rising	Before rising	After rising	Before rising	After walk	Lying covered
1924–1925	°C.	cc.	cc.			°C.	°C.	°C.
Subject VI:								
November 4	1.1	170	172	61	58	32.1	28.1	32.3
November 11	−2.8	164	160	61		32.0	26.7	30.9
November 13		166	173	61	57			
February 5	−11.1	173	175	59	57	31.0	27.7	29.3
February 7	0.0	175	171	55	53	30.1	27.3	30.9
Average		170	170	59	56	31.3	27.5	30.9
Subject VII:								
February 6	0.0	179	180	61	58			
February 10	2.2	176	195	55	53			
February 12	5.6	180	178	58	58			
February 13	−5.6	193	189	61	59			
Average		182	186	59	57			
Subject VIII:								
October 22	4.4	184	176					
February 11	3.3	154	153	59	56			
February 14	−3.3	173	167	64	60	29.2	26.4	30.4
Average		170	165	62	58	29.2	26.4	30.4

not to report the basal metabolism of a number of individuals, nor to discuss particularly the variations in basal metabolism between different individuals nor, indeed, the fluctuations from day to day with the same person. We were primarily interested in the comparison of the metabolism before dressing, and then after rising, dressing, walking outdoors, and returning to the laboratory. The tabular presentation of results (see table 2) has been made upon this basis. Usually each value for the oxygen consumption is the result of two well-agreeing periods. As an index of the temperature to which the young women were exposed when walking out on the campus, the outdoor temperature is noted.

Pulse rate. During the experimental series a large number of pulse rates were obtained. The study of the pulse rate was not the primary object, and we are not satisfied with the pulse counts for a strictly comparative study. The main object was to secure the most accurate observations possible with regard to the oxygen consumption. The pulse values, as recorded, have been averaged and are also given in table 2.

Taken by themselves, they have relatively little significance, except that they are perhaps, on the whole, rather low pulse counts for young women. It is striking to note that in general the pulse rate after exercise is lower than before.

The fact that such exercise as is involved in rising, bathing, dressing, walking down three flights of stairs, walking in the open air for 10 minutes and then climbing three flights of stairs, followed by a half-hour of rest, did not increase the pulse rate is striking and almost inexplicable. It must be recalled, however, that walking seems to have the least effect upon the heart rate of any physical exercise of man thus far studied. The fact that the Nutrition Laboratory has noted astonishing increases in metabolism following active walking, accompanied not infrequently by an actual lowering of the pulse rate (Benedict and Cathcart, 1913; Benedict and Murschhauser, 1915; Smith, 1922) makes it seem not unusual, although still difficult of explanation, that the pulse rates of these young women fall off rather than increase. While we are, frankly, not satisfied with the general plan of recording the pulse rates, it hardly seems possible that a repetition of the series with special attention to the counting of the pulse would materially alter the picture. Until evidence to the contrary is forthcoming, therefore, we must maintain that the pulse rate just prior to rising in the morning may be a few beats higher than that when the subject is lying down ready for a basal metabolism experiment, after having walked to the laboratory or office.

Basal metabolism. Because of the relationship frequently noted in the Nutrition Laboratory between the pulse rate and the metabolism and because of the stress laid upon the supposed correlation between the pulse rate and the metabolism in the given individual, one is quite prepared, in view of the lower pulse rate noted after the walk, to look for a decrease rather than an increase in the oxygen consumption following the activity of dressing and walking on the campus. An examination of the average oxygen consumption of each individual subject, however (see table 2), shows that after rising there is an average increase of 6 cc. with subject II, 3 cc. with subject III, 10 cc. with subject IV, 2 cc. with subject V, no change with subject VI, an increase of 4 cc. with subject VII, and a decrease of 5 cc. with subject VIII. There is, therefore, a general tendency for the entire group to have a slightly increased metabolism after rising, although the average increase is usually quite insignificant, save perhaps in the case of subject IV, when the average increase for 6 experiments was 5 per cent. When the *individual* experiments are taken into consideration, the change in metabolism after rising ranges from an increase as high as 21 cc. (with subject II on February 4) to a decrease of 12 cc. (with subject III on February 7).

In the several series with each subject all controllable conditions were

made the same, in so far as possible. Hence the data might be used for a discussion of the day-to-day variations in basal metabolism. Too little is known of the effects of the psychical state to insure uniformity on each day, and perhaps a much larger series should be studied to examine the variations, uncontaminated by transitory external stimuli. It is justifiable to note, however, that with the same person the difference in metabolism before and after rising on the same day is no greater, in general, than is the difference in metabolism before rising on different days.

It is strikingly evident from an examination of the data that the correlation between the metabolism and the pulse rate of an individual, which has usually been noted in experiments thus far made in the Nutrition Laboratory, is not found in the case of these young women. Allowances should be made, to be sure, for the nature of these pulse counts, which were necessarily only incidental observations, but it seems wise nevertheless to enter a word of caution against applying too strictly the correlation between metabolism and pulse rate as heretofore stressed by the Nutrition Laboratory. This restriction, however, we would apply only to rest experiments and since the experiments here reported were for the most part duplicates of each other, perhaps the variability in pulse rate and metabolism is not great enough to bring out their correlation, which is rather strikingly shown when a moderate degree of muscular activity is involved.

Skin temperature. When the body has been subjected to a night's repose in bed, well covered and well protected from the environmental temperature, experience has shown that the skin temperature has a tendency to become fairly even throughout the entire body (Benedict, 1925). In this research measurements of the skin temperature were made by the thermo-couple method (Benedict, Miles and Johnson, 1919) at approximately twenty points on the body. The details of these locations have already been published (Benedict, 1925).

A typical series of skin-temperature measurements made on subject II on February 4, 1925, when the external temperature was −8.3°C., is given in table 3. The first two observations, as tabulated, are on the forehead and cheek. Positions numbered 51 to 56, inclusive, and positions 14 and 19 are on the trunk. Positions 22 and 11 are on the arms, and position 18 is on the hand. Positions 2 and 3 represent the thighs, 9 and 10 the calves, and, finally, positions 7 and 8 the tops of the feet and 24 and 25 the soles of the feet.

The general uniformity in the skin temperature after a night's repose in bed is shown clearly in the measurements obtained before rising. After the subject bathed, dressed, went out into the air, and returned to the laboratory, the temperature survey (which could be made on all but those points over the abdomen) showed very clearly a pronounced fall in the

average skin temperature. These measurements were made immediately after the subject entered the laboratory, following the walk, and had lain on the bed. Following a rest period of one-half hour after the walk and two respiration experiments, which occupied approximately another

TABLE 3

Skin temperatures before rising, after walking outdoors, and after lying again, covered. Subject II (February 4, 1925)

POSITION	BEFORE RISING (AT 16°C.)	AFTER WALK (AT -8.3°C.)	LYING COVERED (AT 16°C.)
	°C.	°C.	°C.
26	29.9	28.3	31.3
101	30.4	23.6	29.8
51	33.1	29.4	31.1
52	33.0		32.9
53	34.1		33.8
54	34.0		33.8
55	33.4		33.1
56	33.7		34.5
19	33.6	32.1	33.7
14	33.6	31.2	34.1
22	29.9	27.9	27.6
11	33.1	28.1	31.3
18	31.1	24.8	27.8
3	33.0	27.2	31.4
2	31.5	26.9	31.9
10	33.0	26.8	32.8
9	33.1	25.6	32.2
7	31.5	27.3	25.2
24	30.6	26.0	24.3
8	30.7	25.9	23.9
25	30.2	25.5	24.5
Average*	31.8	27.3	29.6
Knee:			
a		23.7	26.4
b		23.0	27.0
c		22.0	25.4
d		22.7	24.7
e		24.9	26.0
Average		23.3	25.9

* Omitting positions 52 to 56 inclusive, i.e., the abdomen.

half-hour, i.e., in general one hour after the subject came in from outdoors, the final skin-temperature measurements were made. It is seen in table 3 that these measurements have a tendency to return to the initial values before rising, although in general the average skin temperature is by no means so high as it was prior to the exposure.

The average skin temperature of the whole body is also recorded in table 3. In making these averages the observations on position 52 to 56 were omitted. Since these represent the median line of the trunk, it is unfortunate that the skin temperatures at these points could not have been obtained immediately after the walk. The experience of the Nutrition Laboratory has shown that but a short exposure to a different environmental temperature is so rapidly reflected in the skin temperature that we decided to sacrifice these observations on the trunk in order to make more rapidly the survey of the rest of the body after the walk. The average skin temperature, omitting positions 52 to 56, was 31.8°C. before rising and 27.3°C. after returning from the walk. One hour later, during which time subject II had been lying in the laboratory, lightly covered, the average temperature had risen to only 29.6°C.

Many of the girls wore their stockings rolled down so that the knees were exposed. A study was made of the actual skin temperature of five positions about the knees, avoiding the skin over the patella. Typical series taken immediately after the return from the walk and again one hour later, after subject II had been lying, covered in the laboratory, are also given in table 3. The five points of observations show an everage skin temperature of 23.3°C. immediatedly after subject II returned from the walk. This survey should also have been made before subject II left her bed, as a part of the long series before rising. Probably the temperature of the knees before rising was not far from 30°C. The fall in temperature due to the exposure was therefore approximately 7°C. One hour later, when the subject was lying covered in the laboratory (room temperature about 16°C.), the temperature of the knees had returned only to 25.9°C.

We have incorporated in table 2 the average skin temperatures (omitting positions 52 to 56 and the observations on the knees) for all our subjects, in so far as they were taken. The general picture is precisely that shown by the detailed figures in table 3. Furthermore, it should be stated that the special study of knee temperatures showed uniformly a great fall in skin temperature on exposure, with but a slight increase one hour after the young women entered the laboratory and lay on a bed.

While the actual numerical value of these temperatures may have but little physiological significance, the important point is that there is unquestionably a lower skin temperature after the exposure and doubtless a change in the temperature gradient in the peripheral tissues, which shows conclusively that, as a result of the exposure, there has been a rather large loss of heat, previously stored in the peripheral tissues. The almost insignificant effect of this considerable loss of heat and fall in peripheral temperature upon the basal metabolism is striking, and is another evidence that under ordinary conditions one can legitimately maintain that heat loss and heat production are two independent processes.

CONCLUSIONS

The basal metabolism measured after a night's sojourn in bed with the body well covered, warm and relaxed, is but insignificantly increased in a period following the muscular exercise of rising, bathing, dressing, walking in wintry weather for 10 minutes, and climbing three flights of stairs, provided that after such exercise the subject lies clothed and lightly covered in a room at *circa* 20°C. for a period of 30 minutes. This finding would seem to justify making basal metabolism experiments after one-half hour's repose, even if the patient leaves home and goes directly to to the laboratory or hospital. The customary sojourn in the hospital over night, prior to such tests, seems therefore to be unnecessary. While our normal individuals did not react to the muscular work of dressing and walking, observations on patients should be made with this in mind before a definite decision to relinquish a hospital sojourn is made. The economic importance of releasing a hospital bed, which may be occupied unnecessarily, justifies further study of this point with hospital patients.

The exposure to cold results in a noticeable lowering of the skin temperature and consequently, to a certain extent, the temperature of the peripheral tissues, which are not warmed to their original degree during one hour of rest, with the subject covered, after the walk. There has thus been a loss of heat previously stored in the peripheral tissues, and this has not affected the heat production. It seems reasonable to conclude, therefore, that at least under the conditions of these experiments heat production and heat loss are two independent processes.

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SUPERIMPOSED RESPIRATIONS OR CHEYNE-STOKES BREATHING CAUSED BY TRAINING

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Dogs sometimes breathe in two radically different ways at the same time, they may pant and also breathe more slowly (fig. 1). Dogs in ether anesthesia may not actually pant, but they sometimes execute panting-like movements while they breathe at a slower rate. Rabbits, cats, cows, camels and many other animals breathe in a similar complex way at rare intervals; and it is readily observed that when the human attempts to breathe rapidly, as the dog does in panting, he often breathes more slowly at the same time.

A careful, analytical study of graphic records of so called periodic or Cheyne-Stokes breathing appears to show beyond a reasonable doubt that the great majority of the forms of periodic breathing are in reality types of superimposed breathing. Even the small number of varieties which can not be analyzed with a satisfying degree of certainty into two or more forms of respiration, appear to be physically and physiologically the equivalent of superimposed respirations. Essentially the same statements may be made concerning paroxysmal tachypnea; in fact, this appears to be a name for a few relatively simple forms of periodic breathing.

We might make use here of our habitual notion that an animal may breathe while the chest is in either an inspiratory or an expiratory position (fig. 1), but the concept of double, multiple or superimposed breathing lays some needed emphasis on the periodicity of the slow changes. The new concept appears to be conducive to a clearer understanding of many respiratory phenomena. It suggests at once that the respiratory mechanism is complex enough to behave in two or more of its characteristic ways at a time as well as in its several different ways at separate times. To refer again to the Cheyne-Stokes breathing, graphic records of superimposed respirations appear in a great number of forms which seem to be only superficially different. The more rapid of the superimposed respiratory waves may change very little or not at all in depth at the crests and troughs of the long respiratory waves (fig. 1, 3 or 6); they may decrease in depth or even vanish at either the troughs or the crests of the long waves; they may increase gradually in depth during the long inspiratory phase

and die down abruptly the moment the long expiration starts; they may begin suddenly after the long inspiration is partly finished and disappear as suddenly at a corresponding level on the expiratory side of the long wave; they may sink deeply into the long wave and in this way completely obliterate what would otherwise be the graphic record of the long respiration, etc., to say nothing about the changes in rate which occur at times along with the changes in depth of these rapid respiratory waves. In the most spectacular varieties of the so-called periodic breathing the rapid waves decorate each long respiratory wave by appearing crescendo on its inspiratory phase and decrescendo on its expiratory phase.

Periodic breathing, in the sense that this term is commonly used, certainly becomes less mysterious when we consider that one of the chief characteristics of the respiratory mechanism is its rhythmicity or periodicity of action, whether it reacts rapidly or very slowly; normal respiration is also periodic. If two respiratory activities of different periodicities occur at the same time (temporally superimposed), the more

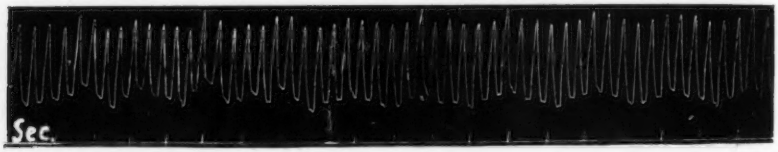


Fig. 1. Respiratory tracing (pneumogram) from a dog which had been very active in the laboratory rooms immediately before it was stood on a table to have its pneumogram taken. Curve rose at inspiration. Rate of rapid respirations (panting), *ca* 156 per minute; heart rate, *ca* 152 per minute.

rapid of these may suffer any one of a great number of possible alterations in rate or depth because of the presence of the slower respirations. In fact, each slow respiration might serve as a special stimulus to re-initiate the rapid respirations that vanished shortly before. Other stimuli, such as tickling the sole of the foot (Kaufmann, 1884), may cause the rapid respirations to continue throughout those stretches of the tracing at which they would otherwise vanish. Apnea may be caused by artificially creating anemia of the medulla, and the state of apnea may then be broken either by a physiological or a mechanical process which restores the circulation through the medulla (Cushing, 1902, 1903), (Eyster, 1906). Respiratory curves obtained by the methods used by Cushing and Eyster show that each apneic period may be terminated by a slow respiration and that the long wave may or may not be decorated with more rapid ones. These long waves often vary greatly with respect to duration and depth in different animals, as well as at different times in a single animal. It seems as if it is the long respirations that are the more sure to occur when the circula-

tion through the medulla is restored. The rapid ones, when they occur, generally begin a short time after either the long inspiration or the long expiration is under way. Some of Eyster's tracings show such temporal relationships between the slow and rapid respirations quite clearly, but he discussed the rapid respirations only.

Working on the assumption that any simple or complex act which an ordinary mammal rarely commits can be caused to occur very frequently if the appropriate method is found to train the animal, attempts were made to train dogs and cats to breathe continuously at two different rates at the same time. It was further assumed that the respiratory mechanism is the only part of an animal which needs the training and that results might be obtained, therefore, while the experimental animal is in ether anesthesia.

The principal observation which proved useful in developing a training method is that anesthetized animals tend to breathe at the same, half or twice the rate at which the lungs are being or have been inflated by mechanical means. Intermediate rates are rare provided the concentration of the ether vapor in the inhaled air is kept constant. When the two respiratory rates are the same, the spontaneous respirations may either coincide with or alternate with the artificial ones and then continue at the same rate after the artificial respiration is discontinued. This rate is often quite different from that at which the animal breathes before the artificial respiration is given. It appears as if the artificial respirations often set the pace for the spontaneous ones.

As a general statement, it may be said that the training method used in the present investigation consists of the acts of mechanically inflating and deflating the lungs at a rate which is much slower than that of the usual respirations of the animal but which is somewhat the same as the rate at which the respiratory paroxysms occur in some of the more spectacular cases of Cheyne-Stokes breathing.

The superimposed respirations presented in the form of tracings represent the most rudimentary (fig. 3 or 6) and the most complex (fig. 4 or 5) types of Cheyne-Stokes breathing that were obtained from the trained animals. It seems superfluous to present the intermediate stages here, because similar ones have been published frequently for several decades. Moreover, although some of the intermediate stages may be more spectacular, due to the fact that the slow waves are more prettily decorated with the rapid ones, they are not quite as instructive as the extreme cases selected.

EXPERIMENTAL. *Methods.* The dogs and cats were anesthetized with ether, a circular opening (ranging from about 3 to 5 cm. in diameter in the cats and about 7 to 10 cm. in diameter in the dogs) was made in the right hand chest wall of each of the animals, a glass cannula with many openings

in the end was inserted through the left chest wall and connected with a mercury manometer to record the intrathoracic air pressure changes, the mouth of a funnel of appropriate size and clear glass was placed securely over the opening in the right chest wall and then the small end of the funnel was connected with a suction pump by means of a rubber tube. (The animals were given artificial respiration via the trachea only as long as the chest was open.) The lungs were then moderately inflated by means of the suction pump, and the rubber tube connecting the funnel and pump was compressed by means of a screw clamp to maintain the normal negative pressure in the chest. The respirations and blood pressure were then recorded on a long smoked paper for thirty or forty minutes before the training was started. The blood pressure tracings were invariably obtained from the left carotid with a mercury manometer.

The original form of the training process involved unclamping the rubber tube to the chest, manipulating a valve of the suction apparatus to give slow respirations of thirty seconds in duration for a period of thirty minutes and then again restoring the normal negative pressure in the chest to watch for superimposed respirations. The two phases of the long artificial respirations were carefully timed and were limited to fifteen seconds each. The lungs were inflated by sucking the air out of the chest, and they were deflated by permitting room air to rush into the chest through the funnel. Three dogs in light ether anesthesia were subjected to this process many times without their showing any definite effects of the supposed training. Further experimentation indicated that the method was faulty in one respect.

The hopes of obtaining results were almost destroyed by the three complete failures, and the fourth dog was accordingly experimented with in a more reckless manner. Instead of taking the pains to accurately time the artificial respirations, the training was done as indicated by figure 2. The respiratory variations in the arterial blood pressure were counted as they were traced on the smoked paper, and the valve in the suction apparatus was so manipulated that each phase of the artificial respirations spanned approximately six of the respiratory variations in the blood pressure. This was done independently of the rate of the spontaneous respirations, which often varied considerably during the training. At the end of the third half-hour period of this training, the dog responded as shown in figure 3. The fifth, eighth and tenth dogs reacted in very much the same way. The reactions of the eighth and tenth dogs were modified by partially destroying the negative pressure in the chest and then later by increasing this negative pressure (figs. 4 and 5). There were no entirely unmistakable evidences that the sixth, seventh and ninth dogs were trained. Two of three cats responded favorably to the training in so far as each breathed at two different rates at the same time after the training ceased.

The seventh and ninth dogs and the one cat which did not respond were probably too deeply anesthetized. The sixth dog was lightly anesthetized, but its respirations were mysteriously jerky and irregular in other respects throughout the experiment; it was already manifesting superimposed respirations of a type. The number of half-hour training periods required for the different animals that responded favorably varied from two to six.

Figure 2, which is inserted to give a better understanding of the improved training method, shows the effects on the blood pressure of artificially altering the intrathoracic air pressure by manipulating the valve in the suction apparatus while the animal breathed spontaneously. These tracings are considered representative ones, but it is realized that exceptions of minor importance occur under certain conditions. In general, the arterial pressure was higher during artificial inspiration than during artificial expiration. It might strike one as being peculiar that there is no sharp fall of brief duration in the blood pressure at the beginning of the artificial inspiration and no sharp rise in the pressure at the beginning of the

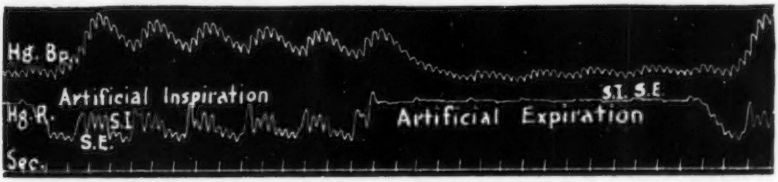


Fig. 2. Intrathoracic air pressure decreased and increased while the dog breathed spontaneously. *Hg.Bp.*, mercury manometer tracing from left carotid. *Hg.R.*, long artificial respiration with spontaneous inspirations, *S.I.*, and expirations, *S.E.*, superimposed on each phase; obtained with mercury manometer from cannulated chest. The small, rapid variations superimposed on *S.I.* and *S.E.* were caused by the heart.

artificial expiration. However, such changes in the pressure were not invariably absent, even though they were never very great as long as spontaneous respirations of moderate depth and rate were in progress. The frequent absence of these initial changes in pressure is a matter which should be considered while studying the later figures, because they are likewise frequently absent when the slow respirations are spontaneous (fig. 3 or 6).

The details of some auxiliary methods employed can be presented to better advantage from time to time later on in the paper.

Slow and rapid spontaneous respirations superimposed. Figure 3 is one of the simplest records obtained from any of the trained dogs. The periodic changes in form of the rapid respiratory waves are certainly too small to be worthy of special consideration. Some of the other tracings obtained from the same animal show various stages of periodic changes in depth and at times in rate as well, but all of the special varieties of Cheyne-

Stokes breathing shown by this dog were only modified forms of the rudimentary type of superimposed respirations here presented. This simple type appears, therefore, to be the one which should be studied most intensively.

The frequency ratio of the slow respirations to the rapid ones was 1:11 plus or minus a small fraction. While training the animal, the effort was made to have each artificial respiration span twelve spontaneous ones (fig. 2), but either the sixth or the twelfth spontaneous respiration was frequently cut short. It appears as if anything which increases or decreases the rate of the rapid respirations also increases or decreases the rate of the slow ones proportionately, for, within surprisingly wide limits, the ratio of approximately 1:11 persisted when the rate of the more rapid respirations was either intentionally or accidentally altered. The rate was altered intentionally by changing the concentration of the ether vapor in the inhaled air and incidentally by certain other means which will be stated shortly. The rate also changed at times when the reasons could not be detected.

The slow waves in the blood pressure and respiratory tracings were not eliminated by opening the abdomen widely, by carefully laying the intestines to one side of the open abdomen, by clamping the right carotid or the abdominal aorta for several seconds at a time or by applying hot, moist towels to the animal to increase its temperature. Each of these manipulations caused the general blood pressure level, the heart rate and the respiratory rate to change more or less, but the ratio of approximately 1:11 persisted. The animal was finally bled to death slowly from the right carotid artery. By the time about one-third of the blood was lost the slow waves in the two tracings were very shallow, those in the respiratory tracing being almost imperceptible. Shortly after this time, very shallow, slow waves could still be seen in the blood pressure, but they could not be detected in the respiratory curve that was obtained with the mercury manometer. However, slow respiratory waves could still be recorded by the special means described in connection with figure 6. All of the slow waves disappeared suddenly when the animal had lost perhaps a little more than half of its blood, and they did not reappear when the blood (defibrinated) was returned temporarily to the circulation via the right external jugular vein.

Some other attempts were made, while working with another dog, which responded to the training in very much the same way, to cause the slow waves to cease. A trephine opening was made in the skull over each cerebral hemisphere without losing a great amount of blood, perhaps not more than 2 cc. During the processes of exposing and opening the skull, the respirations increased in rate; and once the slow waves disappeared suddenly and remained absent for about one minute. The dura was later

Fig. 3

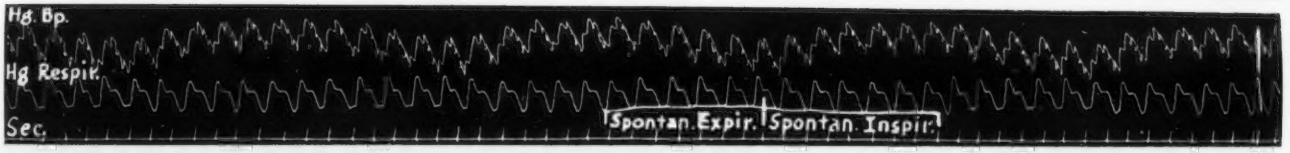


Fig. 4

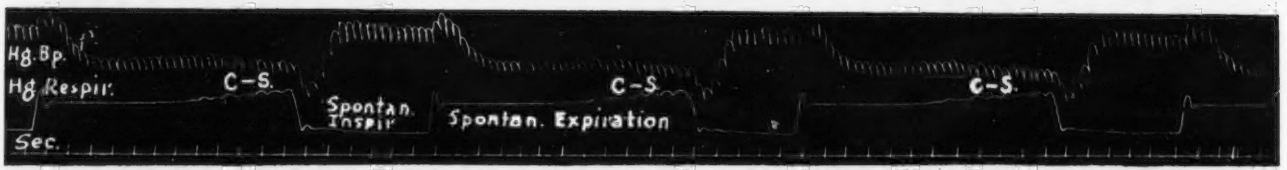
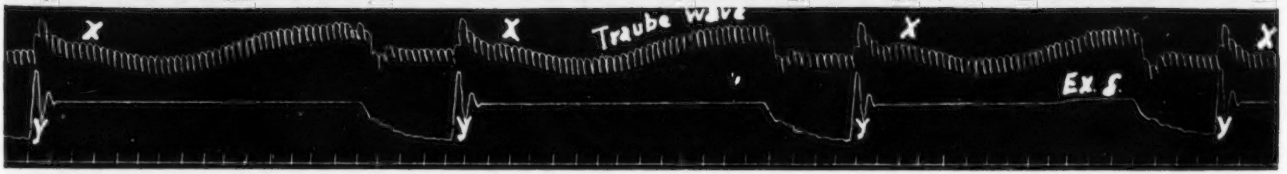


Fig. 5



Figs. 3, 4 and 5. Spontaneous respirations of different rates superimposed. *Hg Bp.*, mercury manometer tracing from left carotid. *Hg. Respir.*, mercury manometer tracing of respirations, obtained from cannulated chest. Figure 3 was obtained from dog 4 and figures 4 and 5 from dog 8. Figures 4 and 5 were derived from a simpler form of superimposed reactions, very similar to those in figure 3, by altering the intrathoracic air pressure after the training was completed. First, the air pressure was increased by about one-half (fig. 4) and then decreased below the normal by about one-half (fig. 5).

Fig. 3. The respiratory curve falls slightly (slow inspiration) as the blood pressure rises and then rises slightly (slow expiration) as the blood pressure falls. About 11 rapid respirations are superimposed on each slow one. The depth or pressure ratio of the slow waves in the respiratory curve to the accompanying slow waves in the blood pressure curve is *ca* 3:8 when expressed in terms of millimeters Hg.

Fig. 4. *C-S*, ascending phase of the rapid waves, of the Cheyne-Stokes breathing, superimposed on latter part of expiration of long duration. The descending phase of the rapid waves is superimposed on the deep inspiration of long duration. The ratio of the rapid respirations to the heart beats is *ca* 1:2, therefore the pseudo-pulsus alternans in the blood pressure tracing above each inscribed *C-S* and each long inspiration. The dog's chest was changing about three-fourths of the time.

Fig. 5. *X*, inspiratory variation in the blood pressure, a sharp inspiration being visible and audible at *Y*. Note the Traube wave with what appears to be the initial portion of its descending limb mutilated by the long respiratory variation. Note the low blood pressure level during the long inspiration, the continued drop in the respiratory curve during each long inspiration, the rapid respirations superimposed on each long inspiration and the secondary expiration, *Ex.S.* preceding one of the long inspirations. Note, also, the absence of rapid respirations preceding each long inspiration and that there is no pseudo-alternans.

ruptured over both hemispheres so that much of the cerebral fluid escaped. The loss of this fluid appeared to have no effect on the slow waves. Later still, a large portion of the skull was removed from each of the hemispheres. Considerable blood was lost. The process of removing the bone was discontinued as soon as it was observed that the slow waves in the blood pressure and respiratory curves were absent. Two minutes and about twenty seconds later the slow waves reappeared suddenly, starting this time with an unusually deep inspiration. The later ones were about the same as before the skull was opened. The intestinal plethysmogram showed the same sequence of events. It may be useful to mention in this connection that the slow waves in the plethysmogram were considerably shallower but corresponded, crest for crest, with the slow waves in the blood pressure tracing. Soon after the onset of various movements of the legs and body (due presumably to the exposed condition of the cerebral cortex), the slow waves disappeared and did not return.

Still another dog, which responded to the training in a similar way, was experimented with as indicated by figures 4 and 5. It can be gathered from figure 4 (obtained while the negative pressure in the chest was only about one-half of that existing in the normal state) that the unusual form of Cheyne-Stokes breathing shown therein owes its complexity to the fact that three varieties of chest alterations were regularly superimposed. One variety of these respirations was shallow and rapid, one was shallow and slow and one was deep and slow. In some simpler tracings not shown here, the deep, slow respirations did not occur. The result was a typical form of Cheyne-Stokes breathing, the rapid respirations being largest at the crests and smallest (usually absent) at the troughs of the slow, shallow waves.

In figure 5 (obtained from the same dog after the negative pressure in the chest was increased to about one-half above normal), the respiratory curve rises slowly before the beginning of the last deep inspiration only. This shallow expiration, *Ex.S.*, was present in tracings obtained later on, and rapid respirations were eventually superimposed on it. The tracings therefore assumed more and more the appearance of those in figure 4. The ascending phase of the Traube wave was by no means inverted, as in figure 4, but its amplitude was somewhat diminished, possibly because of the presence of the rapid respirations preceding each long, deep inspiration. The general fall in blood pressure during each long, deep inspiration continued to occur as long as the lungs were unduly distended.

The Traube wave was observed to develop as the over-inflated lungs underwent a slow deflation, probably because of the elasticity of the lung tissue. After this was observed, the positive variation, *X*, which had been assumed to be the final portion of the Traube wave, was investigated carefully and was found to be associated with a rapid, shallow, visible and

audible inspiration occurring immediately after or really terminating the powerful expiratory stroke. The dip, *Y*, in the respiratory curve is evidently due partly to the mechanical rebound of the mercury column and partly to the brief inspiratory act. This brief inspiration failed to occur in a few instances. In its absence, the respiratory curve stood at a higher level during the long expiratory standstill of the chest, the positive wave, *X*, did not appear and the brief expiratory rise in the blood pressure was somewhat greater in duration. It was further observed that when no respiration occurred to interfere with the Traube wave, this wave rose as shown in figure 5 but did not fall as rapidly. Instead, the blood pressure remained high for ten or twelve seconds and then fell more slowly than it rose. This corresponds better, too, with the original description of the wave by Traube (1871).

Traube did not bring the lungs to a condition of relatively permanent over-inflation by the method used in this experiment, but he probably accomplished the same end by another means. He gave curarized animals artificial respiration by forcing air into the lungs at a moderate rate, suspended the respiration and then observed some long waves in the blood pressure, only the first one of which is being considered at present. Now observations made through glass windows in chests of animals show that if the air that is forced periodically into the lungs does not escape very rapidly through the trachea, each new inflation is superimposed on an already existing one. If the lungs remain in this over-inflated state very long, some of the air in the chest cavity seems to disappear, distended lung tissue taking its place. Then when the artificial respiration is suspended, the over-distended lungs tend to collapse but can do so only slowly because the abnormal negative pressure in the chest decreases slowly.

A detailed consideration of this wave is appropriate here because the phenomenon is an after-effect of a number of respirations (one or more), and it may start before, simultaneously with or after a later period of respiratory activity, as in many forms of Cheyne-Stokes breathing. In connection with this consideration, the following belief expressed by Eyster (1906) becomes especially interesting: "The waves of blood pressure cannot be regarded as a mechanical effect of the periodic respiratory activity; on the contrary the latter must be due to the changes of blood pressure, or both phenomena may be referable to a common cause." In seeking the cause of the long waves of blood pressure, one should first look back to the preceding dyspneic period and then note the ways in which the after-effect of the respiratory paroxysm is directly modified by the group of respirations that occurs at or about the same time as the delayed effect in the blood pressure curve. In connection with Eyster's work it should be noted that a primary and very frequent effect of increasing the intracranial tension is a strong, prolonged contraction of the chest. As to whether this can be

detected in the tracings often depends on how the animal breathes next, whether it first relaxes the chest or breathes with it still contracted. If the chest is contracted down onto the lungs, some of the air in the chest should ultimately disappear similarly as when the lungs are over-distended to fill the chest cavity.

With the possibility in mind that the Traube wave could be due to a slow vasomotor reaction which could be due in turn to an anemic condition of the medulla which could be due in turn to the intracranial pressure being greater than the mean arterial pressure as determined from the femoral artery (Eyster, 1906) (the regulatory mechanism of Cushing, 1903), small trephine holes were made at different places in the skull and the dura ruptured so that much of the cerebral fluid escaped. The data obtained from this dog, and another one which was subjected to the same treatment, showed that the Traube wave was probably not associated with intracranial tension. At least, the loss of cerebral fluid appeared to have no effect on the wave. A lumbar puncture and the withdrawal of 2.3 cc. of fluid from this region also appeared to have no effect on the wave. The puncture was made quite late in the experiment.

Other reactions associated with the Cheyne-Stokes breathing of the trained animals. Special reactions of the eyes were not observable in two of the dogs, and they did not invariably take place in the other animals. It was observed in some cases that the pupils were wider and reacted better to light during the periods of exaggerated respiratory activity than at intervening times. It was also found in some instances that the eyeballs made oscillating movements in various directions. The movements of the two eyes were synchronous, and they were observed more frequently during relative and absolute respiratory pauses.

When the animals breathed in the same way or in ways similar to that shown in figure 3, the rubber tube, which connected the small end of the funnel on the right chest wall with the suction apparatus, was observed to move away from the animal during each short inspiration and to fall slowly toward the dog's head during each long inspiration. The tube also vibrated rather rapidly from the beginning to the end of the long inspiration. Direct observation showed slight tremors of the unbound hind legs and barely perceptible twitchings of the neck, intercostal and rump muscles of two of the dogs. These tremors occurred during the long inspirations only. In the other animals it appeared to be the intercostal muscles alone that twitched. They were often difficult to perceive and could be felt better than seen. The vibrations of the rubber tube were evidently due to the reactions of the intercostal muscles, for they could not be produced later by artificially distending the chests of other animals.

The rubber tube was about one meter in length, and, with the purpose in mind of using it to record the long respirations, it was regulated so that it

formed such a sharp arch in passing to the suction apparatus that it was barely able to remain upright. The arched tube made an angle of approximately ninety degrees with the body of the dog. A thread was attached to the screw clamp, which closed the tube at the top of the arch, this thread was then passed over a small pulley and the other end of it was attached to a muscle lever. The funnel and clamp were pushed away from the dog by each expansion of the chest. The writing point of the lever accordingly rose at inspiration and fell at expiration. As the clamp was pushed farther and farther away from the animal, the tube tended to fall over to one side, toward the dog's head. The ordinary inspirations were not sufficient to cause the tube to fall very far, but these plus a long inspiration caused it to become more and more unstable until it was on the verge of falling completely over when the long inspiration ended. The tube

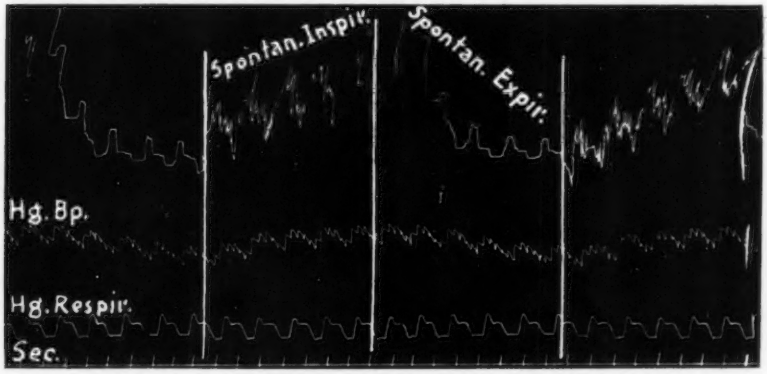


Fig. 6. Upper tracing, superimposed respirations recorded by means of a muscle lever attached to the unstable, arched tube connecting the suction pump with the funnel on the dog's chest. The other tracings are very much like those in figure 3.

returned to its more stable position during the long expiration. The apparatus was accordingly such that it had a two-dimensional action and that it greatly magnified the shallow respirations primarily (fig. 6, upper tracing). This method was applied only after the training was completed and the suction pump was no longer in action.

Two cats which responded to the training by showing superimposed respirations but extremely shallow, if any, long waves in the blood pressure tracings, did not show tenseness of the skeletal muscles and did not cause the rubber tube to vibrate. It appears from this as if it is the muscular tenseness which is responsible for the ascending limb of the Traube-Hering wave. There seems to be a strong tendency for the skeletal muscles, especially the intercostals, to become tense during long inspirations; but

this is only a tendency, for results show that muscle tenseness and inspiration do not invariably occur together.

It is especially interesting in this connection that all atempts to cause simultaneous, slow waves like those in the blood pressure and respiratory curves of figures 3 and 6, by artificially regulating the intrathoracic air pressure, resulted in very poor imitations. They were poor in the sense that the slow waves in the blood pressure were always smaller than expected. For instance, when the artificial reduction of the intrathoracic air pressure amounted to 3 mm. Hg (approximately that indicated in fig. 6), there was only a barely perceptible change, and at times none at all, of the blood pressure level. This was probably due to the failure of the muscular tenseness to appear when the air pressure was artificially altered.

Discussion. Setting the pace for spontaneous respirations, the persistence of the rate ratio of 1:11, the Traube-Hering wave and the Traube wave were reported as found and were discussed only enough to suggest that some important relationships exist between these phenomena and the major phenomenon of superimposed breathing. Statements of belief concerning the probable physiological principles underlying the reactions just stated were deferred until this time. A primary consideration in this discussion is that the respiratory center is multiple in nature, as Lumsden (1923) and others have shown.

Setting the pace for spontaneous respirations of anesthetized animals by mechanically inflating and deflating the lungs may be assumed to mean that the portion of the complex respiratory center which is potentially able to function at the same rate at which the artificial respirations are given, tends, more so than any of the other portions, to react at its characteristic rate before as well as after the artificial respirations cease. The functional units or sub-centers of the general respiratory center which are best fitted to react along with the artificial respirations are those with such characteristic rates of reaction that they are interfered with least by the artificial disturbances in the chest. The least amount of interference seems to occur when the spontaneous respirations take place at the same rate as and either coincide with or fall half way between the artificial ones. Under ordinary conditions, one portion of the general respiratory center usually functions at a time; it probably inhibits action of the other functional units. Under certain conditions, however, two or more of these units may persistently function together and give rise to such superimposed respirations as are herein reported, as well as to many other types of so-called periodic breathing.

Each unit of the respiratory center appears to be able to react at a definite rate only when the conditions remain constant. If the conditions vary, as when the concentration of the ether vapor in the inhaled air is

altered, the rate changes, and the characteristic rate of each of the other sub-centers changes proportionately in the same direction. This is the interpretation laid upon the observation that the rate ratio of 1:11 of the superimposed respirations remained remarkably constant when various things were done to the animals to change the respiratory rate.

A long, shallow inspiration may not cause a perceptible rise in the arterial blood pressure, but, if such an inspiration is accompanied by tenseness of some skeletal muscles, the blood pressure may rise during the long inspiration and fall during the long expiration. This slow change in the blood pressure is a respiratory variation caused by this particular type of respiration. If the slow wave has other respiratory variations superimposed on it, it is a Traube-Hering wave; otherwise, it is a type of Traube wave. It seems probable that the tension of the skeletal muscles impedes the circulation in many of the capillaries and thereby increases the arterial pressure. It is conceivable that the tenseness of the muscles is a result of slow vasomotor reaction, but this might be only a hazardous guess.

When the lungs are over-inflated for some time by forcing air into them, some air disappears from the chest cavity, giving way to the distended lungs. After the force that distended the lungs is removed, the lungs return slowly to their normal condition of inflation. They tend to do this because of their own elasticity, but the rate of deflation is determined largely by the rate at which air returns to the chest cavity to replace the amount previously lost. Of course a similar situation exists when air is sucked out of the chest cavity. Also, a similar situation exists when the chest walls contract down on the lungs for a considerable time. In this last case the compressed lungs eventually return to about their normal state as air disappears from the chest; and when the walls of the chest are later lifted, the lungs enter into a state of abnormal distention which they depart from as slowly as air reënters the chest cavity to take the place of the lost air. The pulmonary circulation is probably impeded when the lungs are in either a state of abnormal inflation or deflation. The blood pressure is then relatively low on the arterial side. As the lungs emerge from either of these abnormal states, the optimum condition for the pulmonary circulation is gradually approached and the arterial pressure rises. This rise is the ascending limb of the first Traube wave which appears as an after-effect of certain reactions (as in Cheyne-Stokes breathing) or manipulations of the chest. It is not essential here to consider the causes of the descending limb of this wave.

When the Traube wave here considered is a delayed effect of a complex of chest reactions, as in Cheyne-Stokes breathing, it may or may not start at the moment the succeeding respiratory paroxysm begins. If it starts before, it might wrongly be assumed that the increase in blood pressure is the cause of the paroxysm; if it starts later, it might wrongly be assumed

that the paroxysm causes the wave in the blood pressure; and if the two phenomena happen to start at the same time, the erroneous supposition might be made that both have a common cause.

CONCLUSIONS

1. Lightly anesthetized dogs and cats were trained to breathe persistently at different rates at the same time, the various types of superimposed respirations being types of Cheyne-Stokes breathing without being associated with intracranial tension.

2. Tenseness of the intercostal and some other skeletal muscles accompanied the long inspirations of the dogs and caused Traube-Hering waves. This type of reaction of the muscles could not be detected in the cats, and the presence of superimposed respiratory variations in the arterial pressure of these animals was always doubtful.

3. Under certain conditions relating to the negative pressure in the chest, a respiratory paroxysm may cause a Traube-Hering wave as an immediate effect and another slow wave, a Traube wave, as a delayed effect. The succeeding paroxysm may or may not happen to coincide with the Traube wave. This wave often greatly increases the difficulty of determining the relationships existing between the periods of heightened respiratory activity and changes in the blood pressure level.

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A NEW METHOD OF ASSAYING THE POTENCY OF THE FEMALE SEX HORMONE BASED UPON ITS EFFECT ON THE SPONTANEOUS CONTRACTION OF THE UTERUS OF THE WHITE RAT

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Isolated unstriped muscle, under favorable conditions, spontaneously contracts. Blair (1923) has demonstrated that the spontaneous contractions of the white rat's uterus submerged in warm, oxygenated Locke's solution, vary in rate according to the period of the sex cycle, being rapid during interoestrus and slower during pro-oestrus and oestrus. These findings have been confirmed by Keye (1923) and Corner (1923) who used strips from the uterus of the domestic sow. Seckinger (1923), using the oviduct of the sow, found that the tubal musculature contracted more rapidly during oestrus and showed slower contractions in interoestrus. Corner (3) has formulated the hypothesis "that the uterine and tubal contraction cycle represents a mechanism probably peristaltic in nature, by which the ova are transported through the tubes and uterus."

The present investigation was undertaken to determine primarily *a*, whether the difference in the contraction rate of the uterine muscle was ascribable to the influence of the female sex hormone (gestational gland, see Frank and Gustavson (1925)), and secondarily, if this proved to be the case, *b*, whether this variation in behavior of the uterine muscle could be utilized to determine the potency of extracts containing the female sex hormone.

1. For control, a number of white rats in which the stage of the cycle was determined by the vaginal smear test of Long and Evans (1922) and which were used in connection with another investigation were employed (Bonham, 1925). The isolated uterus of these normal controls suspended in oxygenated Ringer's solution showed an average rate of one major contraction every 100 to 125 seconds during oestrus (vaginal smear squamous epithelium), and a rate of from 45 to 65 seconds during interoestrus (vaginal smear containing leucocytes) figure 1.

2. Rats previously spayed, whose vaginal smears showed leucocytes, behaved identically with normal controls in interoestrus, developing an average rate of 52 seconds. This rate we designate as the *castrate norm* (see nos. 1 to 8).

TABLE 1

Record of experiments

NUMBER	DATE	NUMBER OF RAT	DESCRIPTION	RECORD OF CONTRACTIONS (AVERAGE)			DATE OF CASTRATION	INJECTED ON	DOSAGE	VAGINAL SPREAD SHOWED
				Rate	Amplitude	Tone				
1	2/ 9/25	30	Castrate norm	57.7	15.6	1.55	2/4/25	No injection	None	Leucocytes
2	4/24/25	48	Castrate norm	58.0	6.6	1.25	3/20/25	No injection	None	Leucocytes
3	5/20/25	34	Castrate norm	65.3	11.6	1.3	2/12/25	No injection	None	Leucocytes
4	5/25/25	63	Castrate norm	34.5	3.7	0.2	5/21/25	No injection	None	Leucocytes
5	5/25/25	61	Castrate norm	60.0	3.9	0.7	5/19/25	No injection	None	Leucocytes
6	5/26/25	59	Castrate norm	44.0	7.4	1.8	5/18/25	No injection	None	Leucocytes
7	6/11/25	37	Castrate norm	46.5	2.6	0	2/26/25	No injection	None	Leucocytes
8	6/11/25	50	Castrate norm	42.5	2.9	0.6	3/21/25	No injection	None	Leucocytes
9	4/23/25	51	Lipoid from follicle fluid	123.5	13.0	0.7		4/22/25	30 mgm.	Squamous epithelium
10	3/ 5/25	14	Lipoid from placenta	106.75	23.35	2.22		3/ 4/25	37.5 mgm.	Squamous epithelium
11	6/11/25	41	Lipoid from placenta	101.5	16.9	1.1				
12	6/11/25	24	Lipoid from placenta	98.5	20.1	2.2				
13	3/28/25	44	Lipoid from corpus luteum	103.0	34.0	2.15		3/26 and 27/25	122 mgm.	Squamous epithelium
14	4/10/25	27	Lipoid from corpus luteum	116.4	17.0	1.5		4/ 9/25	45 mgm.	Squamous epithelium
15	4/16/25	49	Lipoid from corpus luteum	317.5	18.75	0.8		4/15/25	75 mgm.	Squamous epithelium
16	5/22/25	40	Lipoid from placenta (vaginal smear turned)	62.5	8.2	1.6		5/20/25	37.5 mgm.	Squamous epithelium with leucocytes 5/22
17	2/20/25	22	Lipoid from corpus luteum (vaginal smear turned)	51.0	6.25	1.1		2/17/25	100 mgm.	Squamous epithelium with leucocytes 5/20

3. Spayed rats injected with potent lipoid extracts obtained from follicle fluid, corpus luteum and placenta showed the slow major contractions characteristic of oestrus, during the period when their vaginal smears contained no leucocytes (see nos. 9 to 15). With the first appearance of leucocytes in the smear the rate abruptly changed to that of interoestrus (see nos. 16 and 17).

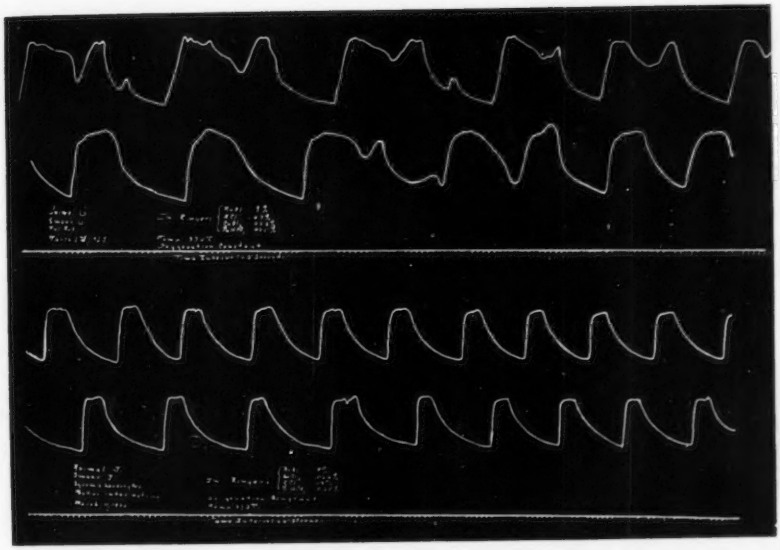

Fig. 1. Above, rat G in full oestrus (spread pure squamous epithelium), rate slow, large amplitude. Below, rat J, immediate post-oestrus (spread shows squamous epithelium and leucocytes). Rate rapid, amplitude less.

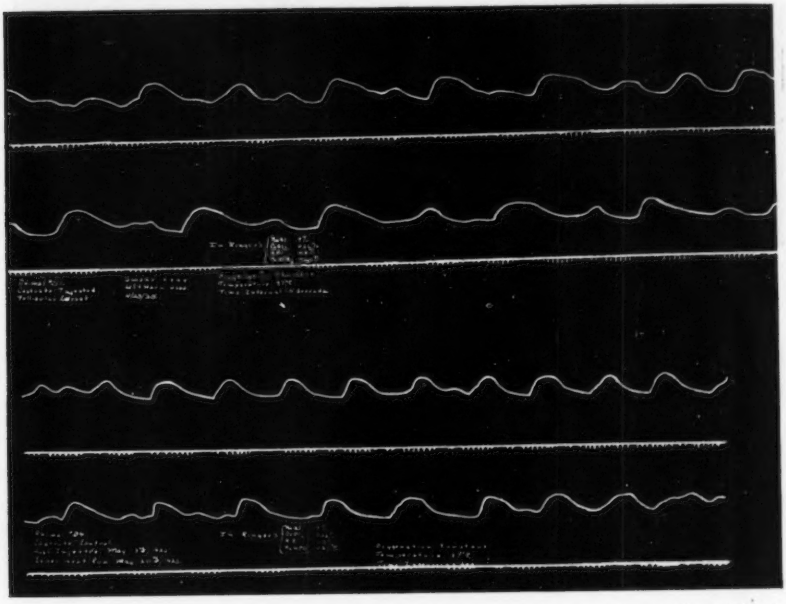

Fig. 2. Above, rat 51, castrate injected with follicle extract; (spread positive), rate 123.5 seconds. Below, rat 34, castrate norm; (spread negative), rate 65.3.

From these results we feel warranted in drawing the following conclusions:

1. That the difference in rate of the spontaneous contraction of the isolated uterus of the albino rat is due to the presence or absence (or subthreshold quantity) of the female sex hormone.

2. That the female sex hormone when secreted in sufficient amount slows the spontaneous uterine contraction rate.

3. That potent extracts whether obtained from follicle fluid, corpus luteum or placenta exert an identical effect.

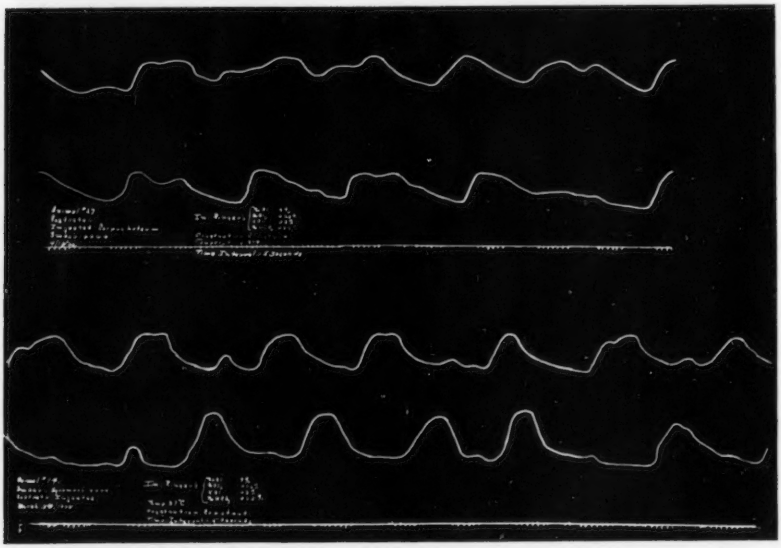

Fig. 3. Above, rat 27, castrate injected corpus luteum; (spread positive), rate 116.4. Below, rat 14, castrate injected with placenta; (spread positive), rate 106.75.

4. That this reaction offers another, exact method for testing and assaying the female sex hormone.

Technic. The rat to be tested was killed by decapitation. The uterine horn from cervix to beginning of tube was rapidly freed from its mesentery and suspended in a chamber filled with Ringer's solution kept at 37° of the following formula:

	per cent
NaCl	0.9
KCl	0.03
$CaCl_2$	0.026
$NaHCO_3$	0.025

A constant stream of oxygen was passed through the solution. After one-half hour had elapsed the contractions were recorded by means of a lever writing on a slowly revolving drum (see records of tracings, figs. 1 to 3).

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ARE REFLEXES FROM THE LARGE VEINS OR AURICLE OF IMPORTANCE IN THE REGULATION OF THE CIRCULATION?

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The manner in which the heart reacts to increased or decreased volumes of blood returned from the veins and the compensating mechanisms which attempt to maintain arterial pressures at a constant level under such conditions have been carefully studied in comparatively recent times. Under conditions of increased venous return, an increased minute volume could be discharged either through an increase in the rate of the heart, or by an augmentation of the systolic output; and either or both of these effects could conceivably occur from a direct reaction of the heart or through effects produced by nervous reflexes. The direct mechanisms which the heart has for adapting itself to variations in blood volume have been clearly established. Patterson and Starling (1914) showed that the greater the return volume, the greater the output during each systole. Wiggers (1921) later extended this conception by showing that with greater diastolic distention the period of systolic ejection is also lengthened. Wiggers and Katz (1922) subsequently showed by volume curves of the ventricles that the velocity of ejection is increased as well. There is no experimental evidence that an increased venous return, of itself, is capable of causing an acceleration by direct action on the heart. Knowlton and Starling (1912) found no change of the rate, in a heart-lung preparation, when the return volume of venous blood is increased. Wiggers (1921) and Wiggers and Katz (1922) working on dogs with the vagi cut, observed that the general tendency of a large saline infusion is to retard the rate, somewhat.

Bainbridge (1915), however, has presented experimental evidence that a *reflex acceleration* of the heart can be caused by an increase of venous pressure. The mean arterial pressure of dogs was recorded, using a damped mercury manometer. The heart rates were counted on records

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made with a Hürthle manometer. His results indicate that the slow or rapid injection of saline or blood causes a cardiac acceleration whenever the venous pressure rises, provided that the mean arterial pressure does not increase to a marked degree. This acceleration is still present if the adrenals be removed but section of the vagus and accelerator nerves abolishes the reaction. Hence, he attributed the quickening of the heart associated with a rise in right auricular pressure to stimulation of afferent fibers of the vagus, resulting chiefly in a depression of the vagus center and to a lesser degree to a stimulation of the accelerator center.

If other factors be constant, these cardiac reactions to increased venous pressure would cause an increased minute output from the left ventricle and an associated rise in aortic pressures. There is evidence, however, that this tendency is counteracted by a number of compensating mechanisms. Eyster and Hooker (1908) showed that with increased aortic pressure a cardiac slowing occurs due to stimulation of the vagal afferent fibers in the aorta. The ultimate heart rate would then depend on the balance of the antagonistic effects caused by stimuli arising in the right auricle and in the aorta. It is possible that a reflex vasodilatation takes place when either the aortic or venous pressures rise, thus tending to maintain arterial pressure at approximately constant levels in spite of an increased minute output. This, however, has not been demonstrated experimentally. McDowall (1924), however, presents experiments on cats to show the converse, namely, that a decreased venous pressure can provoke a reflex vasoconstriction. His interpretations are based on the observation that whenever the venous pressure was reduced, immediate section of the vagi caused, not the usual rise of mean arterial pressure, but a still further fall. This he interpreted as due to the cutting of afferent fibers which reflexly maintain a state of vasoconstriction.

Limitations of mean blood pressure tracings in interpreting cardiovascular reflexes. In studying the part that cardiovascular reflexes play in the adaptation of the circulation to increased and decreased volumes of returning venous blood, it is important to know whether arterial pressure is constant or in which direction it alters. For this purpose, mean arterial pressures recorded by mercury manometers have been generally employed. Actually, a mean pressure does not exist; it is an artificially produced or theoretical pressure. Dawson (1906) has shown, by calculations, that theoretically it is not an arithmetical average of systolic and diastolic pressures but is normally nearer the diastolic level, the exact relationship depending on the gradient of pressure decline during diastole. Further, it may be questioned whether so-called "mean pressures" recorded by a mercury manometer correspond to this theortical mean. Frank (1911) points out that, due to its low vibration frequency, the mercury manometer, even when appropriately damped, may show resonance waves,

or other periodic variations which do not exist in the blood stream. Thus, respiratory variations of pressure are often greater than the cardiac variations, which is contrary to actual pressure relations as established by optical manometers. May it not be possible to have marked variations in actual pressures within the arterial system, during an experiment, and yet record a mean arterial pressure curve which is practically unaltered? This question must be answered before it may be assumed that arterial pressures are really constant when mean arterial pressure shows no variations during experimental conditions.

Furthermore, in the study of cardiovascular reflexes, it is important to have records which distinguish the relative parts played by heart rate, systolic discharge, and peripheral resistance. Such analyses cannot be made from mean arterial tracings. Due to the defects of the mercury manometer, noted above, Frank (1911) states that even the heart rate cannot always be accurately determined; for instance, during labored respiration. Finally, without absolute controls of heart rate, to say nothing of systolic discharge, it is exceedingly difficult to draw conclusions as to reflex vasomotor effects. For this reason, it was deemed important to compare the effects which procedures such as are used in the study of venous reflexes, have on the mean arterial pressure and on systolic and diastolic pressures.

In a series of 24 experiments which form the basis of this investigation it was possible to test these relationships. Mean blood pressure was recorded from the left carotid artery by means of a damped mercury manometer, care being taken that the float followed the changes in mercury levels, exactly. A calibrated optical manometer was inserted into the right carotid artery. The cannula ends of both of these systems were on the same horizontal level. The time when the optical records were taken was marked on the kymograph tracing by an electric signal, so that simultaneous pressure readings could be exactly compared.

In such comparisons, it was consistently noted that the recorded mean blood pressure bears no constant relation to systolic or diastolic pressures, even during any experiment. Results of a typical experiment are charted in figure 1, which has been divided into sections for convenience of description. In section *A*, the control period, the mean blood pressure is seen to lie nearer the diastolic, than the systolic level. In section *B*, where the effects of a saline infusion are shown, the mean pressure rises only 8 mm. Hg, while the systolic pressure is elevated 23 mm. and the diastolic pressure falls by a similar amount. It is obvious that the divergence of systolic and diastolic pressures tends to maintain mean pressure constant and that it can not be said, on the basis of mean blood pressure readings that saline infusion is without effect on arterial pressures. In section *C* during a series of three hemorrhages, the mean pressure is

closer to the diastolic level than it was previously. The mean pressure reflects the change in diastolic pressure more than the alteration in systolic pressure. During a second infusion, shown in section E, the mean pressure, for a time, lies nearer the systolic level, but at the end of the experiment it has approached the diastolic value once more. In the course of the whole experiment, the mean arterial pressure fell from 100 to 83 mm. Hg. The systolic pressure, however, rose from 136 to 146 mm. Hg while the diastolic pressure fell from 76 to 65 mm. Hg. In other words, at the beginning of the experiment the systolic pressure was 36 mm. above and the diastolic 24 mm. below mean pressure but at the end, systolic pressure was 63 mm. Hg above and diastolic 18 mm. Hg below mean pressure. It is clearly seen from the foregoing that mean blood pressure as recorded by a mercury manometer bears no constant relation either to systolic or diastolic pressure and moreover, does not always give an indication of the absolute level or the trend of arterial pressures.

Since such preliminary experiments showed that the injection of saline or hemorrhage cause marked variations of systolic and diastolic pressures which are not mirrored in mean pressure records, it is not possible to exclude reflexes from the arteries and to attribute variations in heart rate solely to changes in venous pressure, when only mean blood pressure records are obtained. For these reasons, we re-investigated the rôle played by reflexes from the veins, recording arterial pressure by calibrated optical manometers.

Methods. Dogs, under morphine sulphate and chloretone anesthesia, were employed. In the majority of experiments the chest was unopened and the natural respirations were recorded by a tambour connected with an intra-pleural cannula. From this system, a side-tube led to a water manometer on which the negative pressure could be read. The chest was opened for a few experiments in which the inferior vena cava was compressed.

Venous pressures were read directly on a saline manometer connected by tubing to a long metal cannula which was inserted into the right auricle from the right external jugular vein. The manometer was filled with saline at intervals and the level at which the flow ceased was read as the correct actual venous pressure. This pressure was corrected, according to the negative intra-pleural pressure existing at the time, and the effective venous pressures calculated. At the end of the experiment the zero level of the venous cannula was obtained by opening the right auricle and all readings were again corrected.

Infusions of saline were given under controlled conditions of temperature and pressure. A three-way stopcock and a T-tube, into which a thermometer was fastened, were connected directly to the jugular cannula. The fluid to be infused flowed through a glass coil, which was

placed in a container filled with water and heated by an adjustable electric plate. By such an arrangement, the temperature of the infusion could be regulated, the infusion given and the venous pressures read from this single system. In some experiments, rectal temperatures were also taken and in others, a thermometer was passed through the lower part of the left external jugular vein and directed toward the heart.

The arrangements for recording the arterial pressures were the same as described in the preliminary experiments. The optical manometer was calibrated, in relation to a base line, by means of a mercury manometer according to the technique described by Wiggers (1924). The calibration from zero to 200 mm. Hg was photographed and from the tracing a scale drawn so that the pressures in any cycle could be read by measuring from the base line. The length of each cardiac cycle was measured on the optical records and the heart rates as shown in the graphs were calculated from this data.

EXPERIMENTAL PROCEDURES. The experimental procedure consisted in studying the effects of saline infusion, hemorrhage, and compression of the inferior vena cava on the heart rate, the effective venous pressure, systolic, diastolic and mean pressures. The procedure was repeated with the vagi cut and similar observations made.

RESULTS. *a. Effects of increased venous pressure on heart rate.* Data regarding the time and volume of venous infusion, before and after vagal section, and the effect of these infusions on heart rate are presented in table 1. It will be seen that, when the vagi are intact, the heart rate increases after an infusion in six experiments (nos. 5, 7, B 18, B 20, B 21) decreases in four (nos. 3, 9, 19, B 19) and remains unchanged in two experiments (nos. 2, 4). After vagotomy, the heart rate increases in four experiments (nos. 15, 17, B 18, B 21) decreases in six (cf. nos. 5, 7, 9, 13, B 19, B 20) and remains unchanged in one (no. 4). As will be seen by further reference to the table, of the six experiments in which an increase in rate occurred before vagus section, it failed to occur in four of these experiments after section but persisted in two. These results lead one to suspect that an increase in heart rate due to a reflex passing through the vagus, and excited by an increase in venous pressure, is less constant than the work of Bainbridge (1915) would indicate.

A critical analysis of typical experiments is, however, the best means of determining the cause of these variations. Referring again to figure 1, section *B*, it will be seen that during a saline infusion the pulse rate increases. In section *E*, after vagotomy, a similar infusion fails to increase the heart rate; in fact, it causes a slight decrease. This experiment, with no further analysis, would appear to support the observations of Bainbridge. However, it is to be noted that in section *C*, when the venous pressure is falling rapidly (before hemorrhage) the heart rate continues

to rise. If the only reason for the increased rate is the greater effective venous pressure, then one would expect the heart rate to decrease coincidently with the lowering of venous pressure. Similar results were shown in other experiments.

A different response is shown in figure 2 where there is a definite increase in heart rate, during infusion, *both before and after section of the*

TABLE 1

EXPERIMENT	HEART RATE (CONTROL)	HEART RATE (INFUSION)	VOLUME OF INFUSION	HEART RATE (AFTER RECOVERY)	HEART RATE (AFTER VAGAL SECTION)	HEART RATE (VAGAL SECTION, RECOVERY, CONTROL)	HEART RATE (SECOND INFUSION)	VOLUME OF INFUSION
2	162–174	165	(?)					
3	128–138	115–128	480 cc.	138				
4	128–140	128–145	(?)	145	162–182		175	
5	142	158	500 cc. in 5 minutes	155	165	165	154	600 cc. in 15 minutes
7	80–100	165	750 cc. in 10 minutes	130	225	210	190	500 cc. in 10 minutes
9	205–210	182–195	800 cc. in 12 minutes	150	225–250	245	225–235	600 cc. in 15 minutes
13	160				207	180	158	650 cc. in 10 minutes
15	114–123	120–135	700 cc. in 5 minutes	136	131	132	137–141	700 cc. in 7 minutes
17	185				203	202	214–219	650 cc. in 9 minutes
19	150	142	700 cc. in 6 minutes	135–141	133	137		
B 18	95–112	124–140	650 cc. in 6¾ minutes	140	155	146	154	650 cc. in 8 minutes
B 19	138–136	120–130	700 cc. in 7½ minutes	127	157	183	156	700 cc. in 6 minutes
B 20	138	138–148	700 cc. in 7 minutes	157	167	170	156–168	700 cc. in 10½ minutes
B 21	110–134	128–143	600 cc. in 5 minutes	105	165	147	163	600 cc. in 7 minutes

vagus nerves (cf. sections *B* and *E*). Another point of difference is that the heart rate decreases as the venous pressure falls after the effects of the infusion have passed off (section *C*).

Thus it is apparent *1*, that an increase of venous pressure does not constantly cause an increase of heart rate even when the vagi are intact, and *2*, when the vagi are cut, there is sometimes an increased rate, sometimes a decreased rate and sometimes no change at all. Therefore, the

evidence does not strongly indicate that a rise of venous pressure produces a reflex increase in heart rate with any degree of constancy or that the afferent path of the reflex lies in the vagus.

b. Effects of increased venous pressure on systolic, diastolic and pulse pressures. We have already pointed out that, during a saline infusion, the mean arterial pressure may remain unchanged while the systolic and diastolic pressures undergo marked alterations (fig. 1, *B*). The most

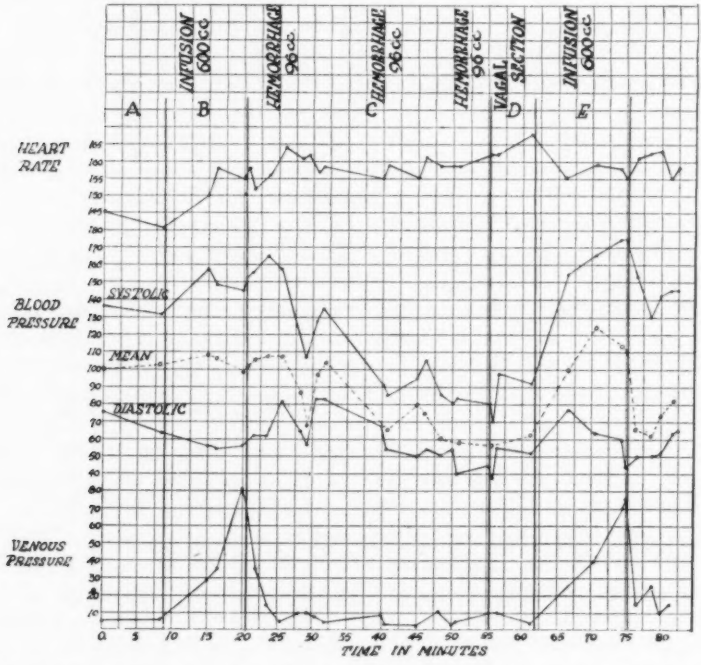

Fig. 1. Graph showing data as to effects of saline infusion and hemorrhage before and after vagal section on heart rate, systolic, diastolic and mean pressures and on effective venous pressures.

significant change noted is an increase in pulse pressure, which, if the rise of systolic blood pressure is taken into consideration, can only mean that there is a greater systolic discharge. This increase in pulse pressure occurred in every experiment, regardless of the alterations in heart rate (cf. fig. 1, *B* and *E;* fig. 2, *B* and *E*). It is evident that while, in some cases, an acceleration may assist the heart in taking care of an increased venous return, the most effective and most constant mechanism is the increase of systolic output. The greater systolic output does not depend

on the integrity of the vagus nerves since it is still present when they have been cut (fig. 1, *E;* fig. 2, *E*).

Our data show that the diastolic blood pressure usually falls (fig. 1, *B;* fig. 2, *B*) and occasionally remains constant, but never rises when saline

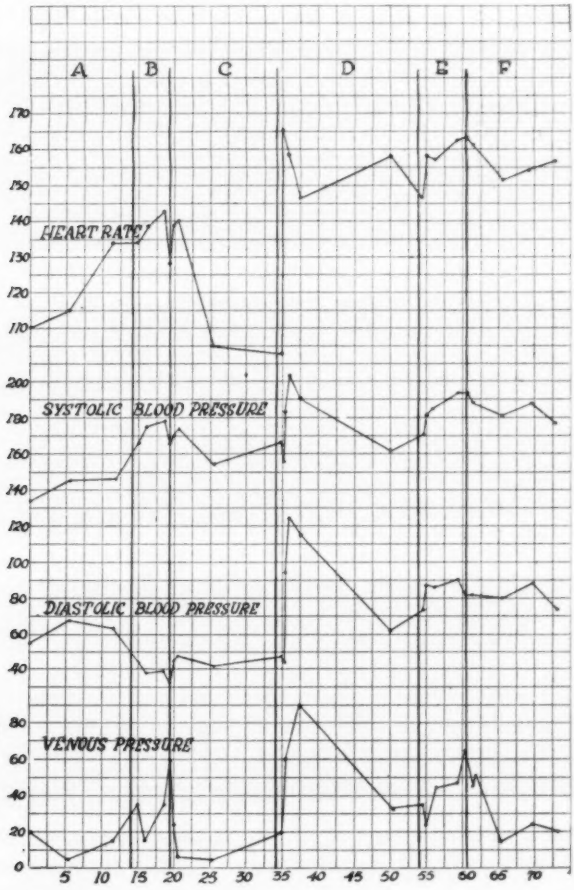

Fig. 2. Graph showing data similar to figure 1. *A,* control period; *B,* effects of 600 cc. rapid infusion; *C,* recovery period; *D,* effects after sectioning of vagi; *E,* effects of a second infusion; *F,* recovery.

infusion is the initial procedure. If a sufficient period is allowed between infusions, the same observation is made. This fall in diastolic pressure might be due to *1,* a decreased heart rate; *2,* a peripheral dilatation, or

3, a decreased viscosity of the blood. The first suggestion is untenable since the diastolic pressure also falls when the heart rate increases (fig. 1, *B*, and fig. 2, *B*). A suspicion that vasodilatation may indeed play a part, is aroused by the observation that the diastolic pressure does not fall, but rises, when saline infusions are given in rapid succession, as in figure 2, *B* and *E*, or when an infusion follows hemorrhage (fig. 1, *E*). One may argue that the fall of diastolic pressure, during a primary infusion is due to vasodilatation and that the rise of diastolic pressure, after a second infusion, or after hemorrhage, is due to the absence of this compensatory dilatation.

It is not necessary, however, to make such assumptions of physiological changes in the caliber of the peripheral vessels; on the contrary, the changes in diastolic pressure are capable of explanation on a simple physical basis. Whole blood is a colloidal solution and as such has a relatively great viscosity. When such a colloidal liquid is repeatedly diluted (as occurs when saline is infused or when tissue fluids return to the circulation after hemorrhage) the viscosity does not diminish to the same degree with each consecutive dilution; on the contrary, the change in viscosity becomes less and less. In other words, a plotted curve, in which the abscissae represent decreasing concentrations of the colloid in per cent and the ordinates the diminishing viscosity, would not be a straight line but would deviate toward the horizontal (cf. Hatschek, 1922). The blood plasma is obviously such a solution and the blood corpuscles as a whole also act in this way as regards viscosity changes (Demming and Watson, 1906).

Applying these facts to the problem in hand it is seen that the viscosity is lessened by appreciable amounts after saline infusion, and that the absolute effect of repeated infusions, at short intervals, or of infusion after hemorrhage, would be less for each successive infusion.

The mechanisms by which the systolic and diastolic pressure changes are brought about during infusion may be briefly discussed. An increased systolic discharge elevates both systolic and diastolic pressures, the effect being less on the latter (Wiggers, 1923). Therefore, a fall of diastolic pressure with an associated increase of systolic output is only possible when the total peripheral resistance is reduced sufficiently to offset the effect of the increased output. During a primary infusion of saline, the effect of lowered viscosity on diastolic pressure may be greater than the effect of the increased systolic output. However, when infusions are rapidly repeated or when infusion follows hemorrhage the viscosity factor becomes less powerful and then the diastolic pressure remains high because of the increased output. To further test this hypothesis, however, a series of experiments were carried out on an artificial circulation schema where each of the various factors could be separately controlled.

In the schema used, the systolic output could be increased or decreased at will and the arteriole resistance was kept constant. Viscosity was altered by diluting the circulating blood with saline, and the other factors concerned in peripheral resistance were kept constant. The schema was driven by a motor so that the rate and the force were uniform. With a

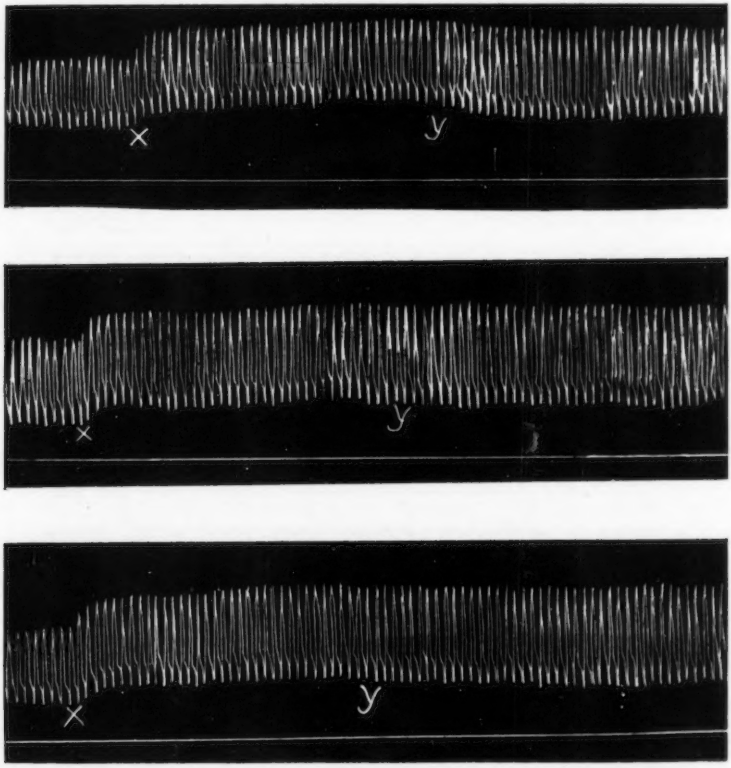

Fig. 3. Three records of systolic and diastolic pressure obtained from an artificial circulation machine showing effects of increasing the systolic discharge, *X*, and the additional effects of repeated dilution of blood with saline, *Y*.

rate of thirty-six beats per minute, an undamped mercury manometer records variations of systolic and diastolic blood pressure with reasonable accuracy. The three tracings in figure 3 were obtained in this way. At the extreme left of each tracing are a few normal beats. At *X*, the systolic output was increased; causing both systolic and diastolic pressures to rise, and the pulse pressure to increase. At *Y*, in the upper tracing,

the defibrinated blood was diluted by about half with normal saline solution. The diastolic pressure falls and the pulse pressure increases further due to the reduced viscosity. At *Y*, in the middle tracing, the already diluted blood was again diluted by half with saline. While a similar tendency of the diastolic pressure to fall is noted, it is obviously

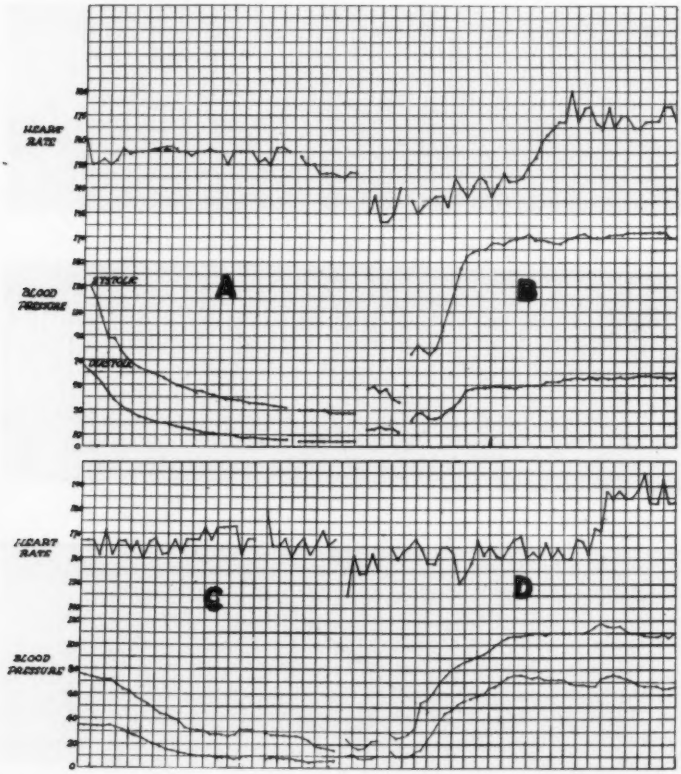

Fig. 4. Plots of consecutive cardiac cycles showing effects on heart rate and blood pressures of *A*, compressing vena cava; *B*, decompressing same; *C*, compressing vena cava after vagal section; *D*, decompressing same.

much less. Finally, in the lower tracing the blood was diluted a third time and here the fall of diastolic pressure, *Y*, is minimal.

Such experiments indicate that the fall of diastolic pressures noted in the animal experiments on the first infusion may be attributed to a decrease in the viscosity of the blood. On the other hand, the failure of the diastolic pressure to fall after repeated infusions or after the dilution of

blood by previous infusion or after hemorrhage, can be explained by a less dominant effect of the viscosity change. Consequently, while these observations do not absolutely exclude changes in the peripheral vessels, directly or reflexly, the results are all capable of explanation on a simple physical basis.

To further test this conception, a series of experiments was carried out in which the inferior vena cava was compressed and decompressed, thus causing a fall of venous pressure comparable to the fall in hemorrhage, and a subsequent rise of pressure comparable to that of an infusion. Such an experiment admits no question as to temperature changes and if there be any change in viscosity it would be an increase rather than a decrease. Observations were made on four animals, two to six compressions and decompressions being made on each animal before and after section of the vagi.

The results were consistent throughout the series, so the analysis of one experiment will serve. The data plotted in figure 4 are the heart rates and systolic and diastolic pressures of consecutive cycles, during the procedure. Figure 4, *A*, shows the fall of both systolic and diastolic pressures, during compression, while the heart rate is almost constant. During the decompression, *B*, both systolic and diastolic pressures rise, but the systolic rise is proportionately greater, the final level being 45 mm. Hg higher than when the compression started. It will be noted that during the decompression the heart accelerates by about 35 beats per minute, the final level being about 16 beats above the rate during the compression. This graph, then, shows an increase of heart rate and an increase of diastolic pressure comparable to the results obtained with infusion after hemorrhage. Figure 4, *C* and *D*, shows a similar compression and decompression after the vagi had been cut. It is seen that the changes in diastolic pressure and heart rate occur as they did previously. Thus, the rise of systolic and diastolic pressures on decompression must be due to an increased systolic output alone. The evidence is clearly against a reflex passing through the vagus, and there seems no necessity for supposing that there is any vasodilatation reflex involved. Mechanical factors alone are a sufficient explanation.

McDowall (1924) presents evidence indicating that a vaso-constriction follows a lessened venous return and that this constriction can be abolished by section of the vagi. In his experiments, venous pressure was decreased by various means such as hemorrhage, injection of histamine, alcohol, etc. He states that if the vagi be cut during the period of lowered pressures, the pressures fall still lower. We have repeated the experiment of sectioning the vagus nerves, immediately after hemorrhage, in seven experiments. In only one did the mean blood pressure fall, and in the other six instances it was unchanged or rose. Figure 5 shows data

of consecutive cycles during the more typical experiments. After section of the right vagus, both the systolic and diastolic pressures fall somewhat and within a short time rise above the initial level. This might seem to

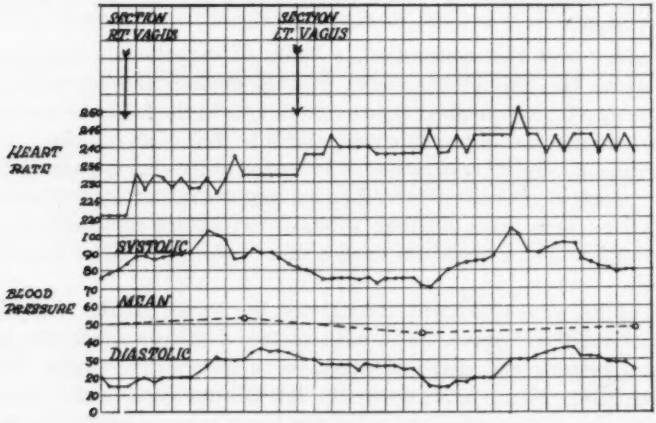

Fig. 5. Plot of consecutive cycles showing effects of right and left vagus section on systolic, diastolic and mean blood pressure as well as on the heart rate.

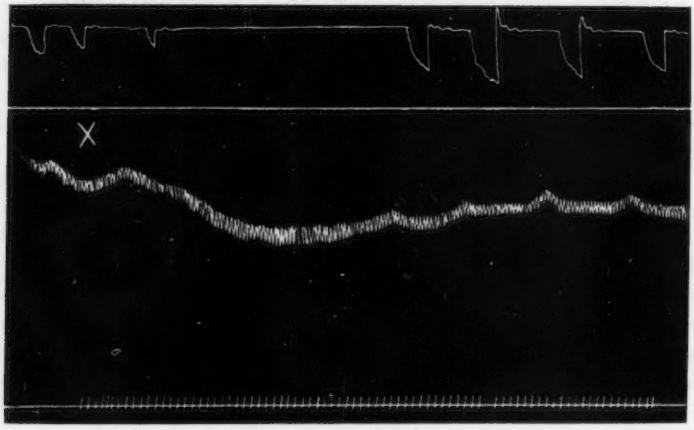

Fig. 6. Mean blood pressure and respiration record from an only experiment where vagus section after hemorrhage caused a fall of mean pressure. Discussion in text.

corroborate McDowall's findings unless the respiratory record is correlated to the pressure records. This shows that a period of apnea supervenes when the blood pressures are falling after the vagotomy, and the

subsequent rise of pressure occurs in relation to a deep respiration. Consequently, it is clear that these changes in pressure are due to mechanical effects of respiration and the effect is more pronounced because, as Wiggers (1912) has shown, the pressor effect of respiration on blood pressure is greater after hemorrhage. Figure 6 shows the only experiment in which a drop in mean blood pressure was obtained by section of the vagi after hemorrhage, that is at all comparable to the published records of McDowall. Both vagus nerves were cut simultaneously at the point labelled *x*. The respiration record written simultaneously shows that this fall of pressure is intimately related to a respiratory pause, which is undoubtedly the cause of the pressure decline. Similar records are obtained when apnea is produced by stimulation of the central end of the vagus nerve (Wiggers, 1912). Thus from our records, we can only associate a further fall of mean arterial pressure following hemorrhage and vagotomy with apnea and find no evidence of the peripheral vessels being affected by reflexes from the right auricle

SUMMARY

Twenty-four experiments were performed on dogs in order to determine to what extent nervous mechanisms come into play, 1, in adapting the minute discharge of the ventricles to the volume of returned blood, and 2, in compensating for the changes in arterial pressures which are thus produced. Comparative studies of accurately determined systolic and diastolic pressures and mean arterial pressures showed that the latter does not faithfully record changes in intravascular pressures and gives no indication of the actual level or trend of pressure changes produced by such experimental procedures as it is necessary to employ. For this reason, calibrated optical records of arterial pressure changes were utilized in evaluating reactions. Changes in effective venous pressure and in respiration were also recorded. In brief, the following are the results which were obtained and the conclusions drawn:

1. In animals with intact vagus nerves, the introduction of saline solution at body temperatures caused an increase in heart rate in approximately 50 per cent of the animals examined. This indicates that an increase in heart rate is a less constant mechanism for increasing the minute discharge than the experiments of Bainbridge would indicate.

2. A similar increase in heart rate occurred in one-third of these animals after the vagi had been cut, as well as in a number of other animals where the vagi had been cut at the start. In many animals the heart rate continued to increase after the venous pressure had fallen after the discontinuance of an infusion.

Furthermore, when the inferior vena cava was compressed and subsequently released, an acceleration of the heart was noted in several experi-

ments, both before and after the vagi had been cut. These results do not support the conclusion that the cardiac acceleration following saline infusion or caval decompression is due to the inauguration of a vagal reflex from the distended veins and right auricle.

3. Irrespective of whether the heart accelerated or not, a first infusion of saline caused an elevation of systolic pressure while the diastolic pressure either remained constant or fell. As the complex of an increased pulse pressure and higher systolic pressure could only mean a greater systolic discharge in those experiments where the heart rate remained constant or decreased, the conclusion is warranted that the increased minute discharge is most constantly brought about by an increase in the systolic discharge.

4. The characteristic fall of diastolic pressure found after a first infusion is replaced by a rise of diastolic pressure on rapidly repeated infusions or after large hemorrhages. The idea that the fall of diastolic pressure on the first infusion may be due to a compensatory vasodilatation which later fails, is discussed and evidence presented to show that it can be accounted for by the decreasing gradient of the viscosity change which follows repeated dilutions of blood by saline infusion or in consequence of hemorrhage.

5. The reduction of venous return and auricular pressures caused either by hemorrhage or compression of the vena cava causes a characteristic reduction of arterial pulse pressure and blood pressures which can be entirely accounted for by a reduced systolic discharge. Subsequent cutting of the vagus nerves causes either a further cardiac acceleration or, what is more common, no change in heart rate but this was not as a rule accompanied by any changes in systolic or diastolic pressure that could be interpreted as a sudden vasodilatation of a reflex nature In one experiment where a moderate fall of pressures followed vagal section this was clearly due to a rather prolonged apnea vagi and was brought about by the removal of the pressor factor of respiration which acts through a reduction of systolic discharge and not on the peripheral resistance. The conclusion is reached that the experimental results do not confirm McDowall's interpretation of reflex vasomotor action inaugurated by a lowering of auricular pressure.

In conclusion the writers desire to express to Prof. C. J. Wiggers their appreciation of the help given them throughout this research.

ADDENDUM. Subsequent to the printing of the foregoing article, a paper by C. I. Reed has appeared (This Journal, 1925, lxxiv, 61) which confirms our work, in that the post-vagotomy fall of blood pressure was not found to be a constant phenomenon when blood pressure was low, thus raising the question "whether the vagi constitute the afferent paths for this hypothetical pressor reflex from the great veins."

We see no experimental evidence, however, to support the further suggestion that "in some animals the main pathway may be in the splanchnic trunks." Nor are we convinced by the experimental data presented that any other vasomotor or cardiac reflex is concerned. On the contrary, we are confident that a careful reconsideration of Reed's records will confirm our interpretation, viz. that the presence or absence of the post-vagotomy fall in blood pressure is entirely contingent upon the variable effects on respiration.

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THE EFFECTS OF PROGRESSIVE ANOXEMIA ON THE HEART AND CIRCULATION

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While the effects *of anoxemia* on the circulation have been studied extensively both in man and experimental animals, a critical interpretation of the data obtained leads to conflicting conclusions as to whether or not an improved circulation plays any part in compensating for the deficient oxygen in the blood and leaves unexplained the mechanisms through which circulatory failure arises when the oxygen percentage in the inspired air reaches a critical level.

The results of experiments on man and animals show that as the oxygenation of the blood decreases during a progressive anoxemia, the heart accelerates, the systolic pressure is maintained or gradually rises, the diastolic pressure undergoes similar changes or gradually falls while the pulse pressure either remains unaltered or increases (Whitney, 1918; Schneider, 1921; Greene and Gilbert, 1921, 1922; and Schneider and Truesdell, 1924). In spite of the statements of Skelton (1921) as to the inaccuracy of the "heart rate pulse pressure" index of minute discharge, the conclusion may still be drawn from these observations that the minute discharge is increased for, as Wiggers (1923, p. 367) has pointed out, Skelton's results on careful analysis "merely confirm what has been generally admitted and emphasized, viz., that no quantitative relation exists. A perusal of the tables shows equally clearly, however, that *without fail* the product of pulse pressure and heart rate changes *in the same direction as the minute discharge.*"

Furthermore, the experiments of Hilton and Eichholtz (1925) indicate that anoxemia produces an improved flow of blood through the coronary vessels, in this way supplying an increased blood flow through the heart even when changes of arterial pressures are not present.

On the other hand, there are distinct evidences in the accumulated data which cast doubt upon the fact that a compensatory increase in blood flow occurs. The peripheral venous pressure slowly declines during anoxemia

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in man, and blood-flow determinations of the arm and hand show a decrease in flow in the majority of subjects examined (Schneider and Truesdell, 1924). Attempts to determine the minute volume and systolic discharge by gasometric methods, though made under extremely difficult technical conditions, indicate that in man and animals the systolic discharge is distinctly reduced, and most of this work also fails to show that the minute volume is increased (Schneider and Sisco, 1914; Doi, 1921; Schneider and Truesdell, 1924). Consequently, many investigators are no doubt ready to conclude with Schneider and Truesdell "that the circulatory changes in anoxemia do not serve as a means of compensating to a lack of oxygen." In formulating such a conclusion, however, it is necessary conveniently to ignore the fact that the sustained or rising systolic pressure and the unchanged or increased pulse pressure which occur with an accelerating heart require a different interpretation.

When the percentage of oxygen in the inspired air is reduced to a point that may be called critical for any given individual or animal, alarming symptoms of circulatory failure develop. The clinical signs of muscular weakness, cold sweat, pallor, disorientation and fainting have been frequently described in man and need no repetition. The circulatory collapse is initiated by a rather abrupt fall of diastolic and systolic pressures and is soon followed by great slowing of the heart due to rhythm and conduction disturbance (Greene and Gilbert, 1921, 1922). These changes are coincident with marked respiratory depression but definitely precede the final cessation of respiration.

Three possible causes of the circulatory failure suggest themselves; 1, failure of respiration with secondary failure of the heart due to asphyxia; 2, primary cardiac depression due to a lack of O_2 in the coronary vessels, and 3, primary failure of the peripheral circulation as seems to be the case in shock. As the circulation begins to fail before respiration ceases the first suggestion is untenable. The last two suggestions therefore remain as possibilities. That circulatory failure may be of cardiac origin is not improbable for in spite of the observation of Evans and Starling (1913) and of Hilton and Eichholtz (1925) that the heart is able to abstract adequate oxygen for its use even when the anoxemia is very great, a certain minimal oxygen content is probably necessary in order to support normal activity of the heart. The observations of Greene and Gilbert (1922) that extreme anoxemia acts directly on the heart to slow the S-A rhythm and to suppress internodal conduction, is evidence that this is true. Whether or not it is by virtue of such slowing alone that the circulation fails has not been demonstrated.

Critically considered there is no real evidence that failure of the circulation is initiated by changes in the peripheral circulation. As nearly as can be judged by available methods the capillary flow remains unaltered

(Schneider and Truesdell, 1924). Inasmuch, however, as the progressive fall of venous pressures and the cardiac acceleration in the pre-critical stages as well as the clinical symptoms and abrupt decline of arterial pressures are somewhat similar to those observed in shock, this possibility cannot be entirely disregarded.

Since, therefore, the many gaps in our knowledge leave us with an imperfect picture as to how the heart and circulation are affected during moderate and marked degrees of anoxemia it seemed desirable to make a further attempt to investigate the subject by methods that have as yet not been applied to the problem.

Method. Two groups of experiments were conducted on dogs under chloretone and morphine anesthesia. In the first group, the reactions were studied on naturally breathing animals. The trachea in each case was connected through a set of inspiratory and expiratory valves with a 25 liter respirometer of the type described by Burlage and Wiggers (1925). This recorded the rate and depth of respiration directly on a kymograph. During inspiration, the animal drew air from the tank through an inspiratory valve and during expiration the air was returned to the tank through two soda lime bottles which absorbed the CO_2. To compensate for the decreased volume of the respirometer due to the renewal of oxygen, a slow stream of nitrogen was introduced from time to time. Air samples were taken from the inspiratory tube and analyzed for CO_2 and O_2 in the Haldane air analysis apparatus.

In these experiments, systolic and diastolic pressures and the changing contour of the aortic pressure pulse were recorded by inserting an optical manometer, of the type recently described by Wiggers (1925), into the carotid artery and subsequently calibrating it. Records such as are shown in figure 3 were obtained at intervals of five minutes or less. From these records, values for the duration of systolic ejection and heart rate were also obtained. In addition, heart sounds were recorded from the precordial region of the shaved thorax by means of the sound capsules of Wiggers and Dean (1917). From these records the duration of total systole and heart cycle were calculated. The difference between the total systole and ejection phases thus calculated represents the isometric contraction phase.

In a second group of experiments, the chest was opened and the lungs were inflated by air from the respirometer by means of a motor-driven pump. The natural collapse of the lungs returned the expired air through a soda lime bottle to the tank, thus establishing a closed circuit.

In these experiments simultaneous tracings of left intraventricular and aortic pressures were recorded every five minutes or less by calibrated optical manometers, according to the technic developed by Wiggers. Typical records are shown in figure 5. In such tracings all the changes already described were determined from the aortic tracings while the

intraventricular pressure curves were also analyzed as regards changes in initial tension, changes in gradient and contour. In both series of experiments, changes in effective venous pressure were followed by taking readings every five minutes. The technic described in a previous paper (1925) was used, for animals with closed chests.

Results on healthy animals during natural breathing. Fourteen experiments were carried out using the rebreathing apparatus already described. Under the conditions of our experiments the oxygen percentage was reduced to about 7 or 8 per cent in about one and one-half hours. At this rate of deoxygenation, distinct evidence of circulatory failure, referred to as the crisis in this paper, began when the oxygen of the inspired air had decreased to 9 or 10 per cent but in a few instances the critical level was lower (e.g., in experiment 17 when it occurred at 5½ per cent). A tabulation of the general results was made but as they were so consistent a few detailed charts of the course of events will serve to illustrate the general character of the circulatory changes found in all. Figure 1 shows typical changes in animals with vagi intact and figure 2 results in animals whose vagi had been divided.

Heart rate. As found by previous investigations, the heart rate progressively increased up to and beyond the crisis, in all but one experiment. This is well shown in the chart of figure 1 in which the rate increased from 65 to 190 beats per minute. The absolute increase was determined by the initial rate at the time rebreathing started. When the vagus nerves were sectioned previous to rebreathing, the normal increase in rate was much less and was delayed until a considerable decrease in the oxygen had taken place (fig. 2). This is in agreement with observations of Greene and Gilbert (1922) and indicates that the early acceleration is caused by a vagal depression, while just prior to the crisis the accelerator mechanism is stimulated.

At the crisis and for some time after, the heart rate remained rapid and then a quick decline in rate occurred, regardless of whether the vagi were intact or cut (cf. figs. 1 and 2). This was also noted by Greene and Gilbert (1922) and as in their cases, was probably due to depression of the S-A node and to disturbances of conduction.

The fact that these observations accord with those of other investigations makes it probable that the general reactions of animals under our conditions of experimentation were similar to those in other laboratories.

Venous pressure alterations. Henderson and Barringer (1913) have pointed out that the filling of the ventricles and the consequent volume of systolic discharge depends not on the absolute but on the effective venous pressure. As the depth of respiration changes during anoxemia, the attendant changes in intrathoracic pressure may not affect the actual pressures in the central or peripheral veins to the same degree as it does the

effective pressure. Consequently, changes in peripheral venous pressure, such as have been obtained in man during anoxemia should be interpreted with caution when the depth of respiration changes. It is obvious that they may not be compared directly to our data of venous pressures corrected for intrathoracic pressures obtaining at the time.

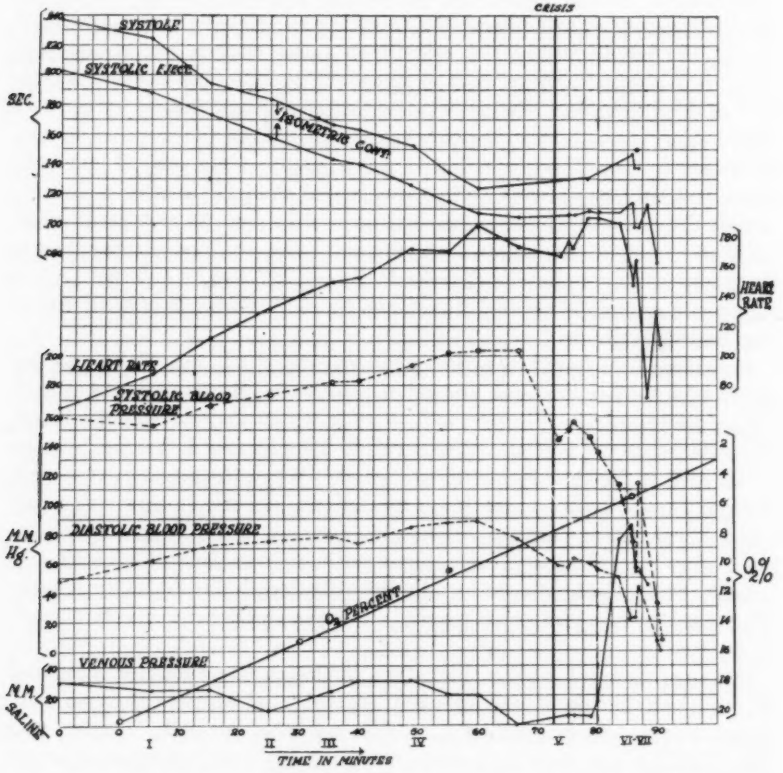

Fig. 1. Chart showing data during course of a progressive anoxemia. Vagi intact; natural respiration. Description in text. Roman numerals at bottom correspond to optical tracings shown in figure 3.

The changes in effective auricular pressure observed in these experiments are shown in figures 1 and 2. They corroborate in a general way observations on man up to the crisis, i.e., there was a slight tendency for effective venous pressure to decrease up to this time. In no case was the drop profound such as is typical of experimentally produced shock (Wiggers, 1918). In some cases, the effective venous pressure showed considerable

variation, the curve first falling and then returning to its normal value (fig. 1). In the majority of cases there was no further change until the crisis (fig. 2) but in two experiments a further fall occurred 10 to 20 minutes before the crisis, as is shown in figure 1.

At the crisis, the effective venous pressure began to rise and thereafter continued to elevate until it reached very high levels. In fact, the eleva-

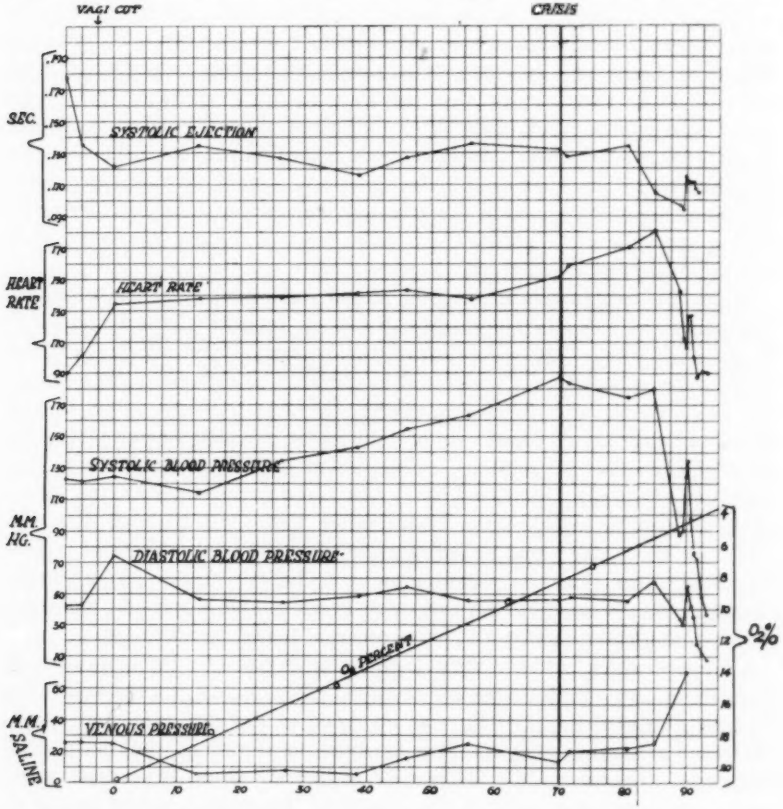

Fig. 2 Chart showing data during course of progressive anoxemia. Vagi cut: natural respiration. Description in text.

tion of effective venous pressure is one of the most definite criteria for the beginning of the crisis.

These observations indicate that, up to the crisis, the reduction in the amount of blood returned to the heart is not serious. If any accumulation of blood in the peripheral vessels occurs it is reasonably counterbalanced by other mechanisms favoring the return of blood (e.g., deeper respiration).

The abrupt rise of venous pressure at the crisis absolutely precludes a sudden failure of the peripheral circulation or venopressor mechanisms but, on the contrary, strongly supports the belief that it is a passive congestion due to a reduced minute output of the heart. Further, as this occurs before the final slowing of the heart, we have presumptive evidence that the contractile power of the heart is also affected.

Systolic and diastolic pressures in progressive anoxemia. During the early stages of anoxemia, the systolic pressure rose consistently and considerably (figs. 1 and 2). In many experiments, there was an accompanying rise of diastolic pressure, but this was by no means as constant; in fact, in three experiments, it fell slightly. In all cases, the pulse pressure tended to increase considerably. At this point, attention may be called to the combination of an increased pulse pressure, higher systolic pressure, increased heart rate, and a slightly lowered effective venous pressure. Such a combination of events occurred in most of the experiments and is well illustrated in figures 1 and 2.

There are two possibilities which might explain such a combination: First, that there is a larger systolic discharge, in spite of the faster rate. Wiggers and Katz (1920) have shown in an experimental study on the effect of epinephrin, that this is possible. However, it is difficult to harmonize this with the slightly decreasing effective venous pressure unless this is associated with an increased rate of ventricular relaxation or an increased vigor of auricular contractions. The second possible explanation is the combined effect of an increased heart rate and decreased peripheral resistance. A cardiac acceleration alone raises both systolic and diastolic pressures, the latter being raised more, while decreased peripheral resistance lowers the diastolic pressure more. The two forces acting simultaneously might result in an increased systolic and a lowered diastolic pressure, and consequently the pulse pressure might be increased.

A differentiation between these two factors is given by the contour and gradient of the arterial pressure records. At equivalent pressures, the diastolic gradient may be taken as an index of the rate at which blood leaves the arterial system and thus becomes a criterion of total peripheral resistance. Tracings I, II, III and IV of figure 3 show cycles from successive tracings taken early in the course of the anoxemia and during a period when the diastolic pressure was almost constant. Aside from the change in rate, one sees that, in each successive record, the fling of the primary wave, P, becomes greater and a steeper gradient, G, develops in the early diastolic period. A straight edge applied to the descending limb shows that the gradient becomes somewhat steeper, the actual change of the angle being from 23° to 35°. Therefore, the contour of these curves indicates that there may be a slight decrease in total peripheral resistance which could result from dilatation of either the arterioles or the capillaries, or from a possible change in viscosity induced by the anoxemia.

We may conclude, therefore, that as long as the heart accelerates it is not necessary to assume an increased systolic discharge in order to explain the changes in pulse pressure or blood pressures. On the other hand, there is evidence that the peripheral blood flow is accelerated by changes in the peripheral resistance.

In the experiments, however, where the heart rate remains constant or becomes slower (e.g, figs. 2 and 6) and yet systolic pressure increases and pulse pressure becomes larger we can only infer that the systolic discharge has increased, in spite of the fact that effective venous pressures are slightly less.

A critical analysis of the blood pressure data therefore indicates that, contrary to the interpretations of other investigators, any or all of three factors may be operating to increase the volume of flow through the body, viz., 1, an accelerated heart; 2, an increased systolic discharge, and 3, a decrease in peripheral resistance.

At the crisis, distinctly different effects on the systolic and diastolic pressures appear. As shown in figures 1 and 2 the first change is a fall of systolic and diastolic pressures, the systolic dropping to a greater degree. As this often takes place while the heart is still rapid, and as a marked increase in venous pressure occurs at this time, it suggests that the myocardium is failing. Optical tracings taken at the crisis and shortly after are shown as segments V and VI of figure 3. Such records show that the heart rate remains fast, that the pulse pressure is small, and further, that the decline of pressure occurs almost entirely during the latter part of systole, the diastolic gradient becoming practically horizontal. Such changes can only mean a reduced systolic discharge.

In the lethal stages the heart rate slows and often becomes irregular while the pulse amplitude reduces progressively (fig. 3, VII, VIII). Sometimes, rates as low as 20 or 30 per minute suddenly develop suggesting heart block; quite often an alternation also appears (fig. 3, VIII). As apnea develops, the pulse pressure becomes very small, but even in this extreme condition a deep respiration causes both systolic and diastolic pressures to rise temporarily. While evidence of grave cardiac depression occurs before the cessation of respiration, the heart always continues to beat after respiration has ceased.

It is obvious from such observations that the real circulatory failure which rapidly develops at the crisis is due to a cardiac depression, and only later is enhanced by slowing of the heart.

It was desirable, as a next step, to determine if possible the precise nature of the changes occurring in the heart during the course of a progressive anoxemia. To this end, the changes occurring in the duration of the isometric contraction and systolic ejection phases were first investigated in naturally breathing animals.

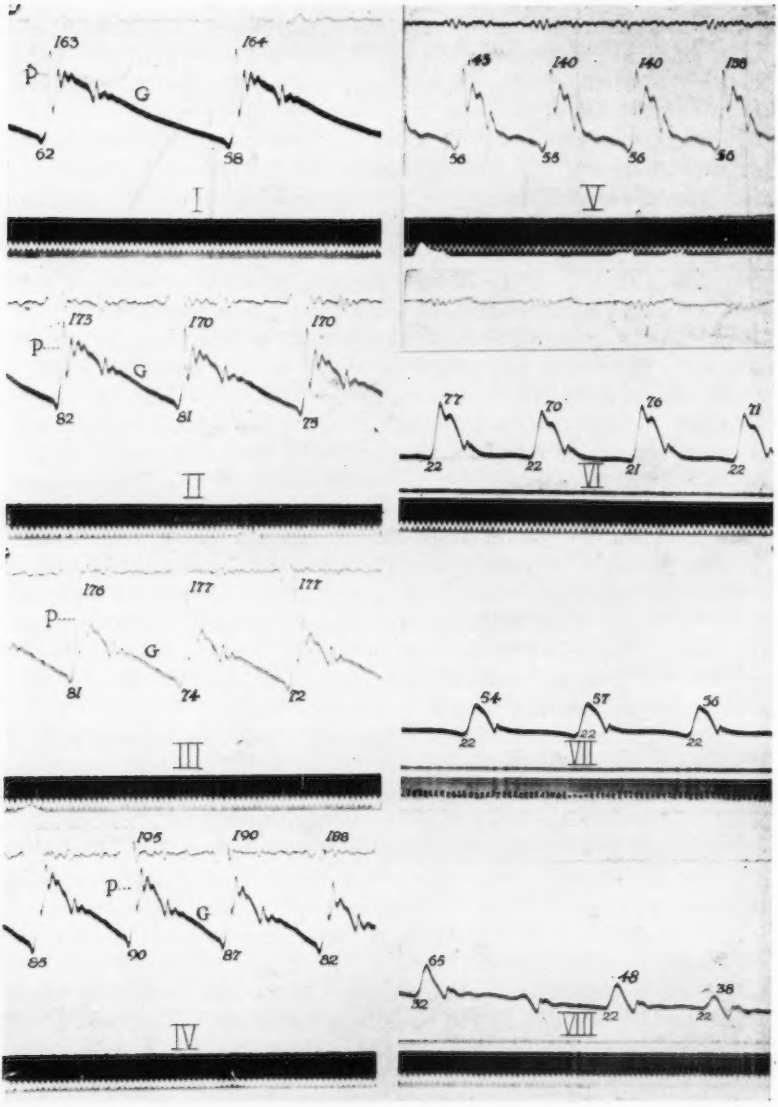

Fig. 3. Segments of optical records showing heart sounds and carotid pressure changes during course of progressive anoxemia from same experiment as chart of figure 1. Numerals on arterial records indicate values of systolic and diastolic pressure, determined from calibrated manometer. Base lines in segments I, II, III, IV, V merge with top of block at bottom. Description in text.

The isometric contraction phase in progressive anoxemia. In figure 1 the values for the duration of systole, determined from heart sounds, and the length of systolic ejection, calculated from arterial pressure records, are plotted at various intervals during a progressive anoxemia. The vertical distance between any two corresponding readings gives the length of the isometric contraction phase. A study of such charts from 14 experiments shows results similar to those plotted in figure 1, i.e., the isometric contraction phase decreases up to the crisis. Inasmuch as such changes in heart rate, diastolic pressure or venous pressure as are found in these experiments do not of themselves induce such an abbreviation of the contraction phase (Wiggers, 1921), this suggests that a mild anoxemia may in fact increase the rapidity of ventricular contraction.

After the crisis, the isometric contraction phase more or less abruptly but always definitely lengthens in ten experiments (e.g., fig. 1). The fact that this occurs in spite of a rapidly falling diastolic pressure and an increasing venous pressure (both of which have a tendency to shorten the phase according to the results of Wiggers, 1921) strongly indicates that the rapidity of ventricular contraction is decreased, thus supplying at least one of the possible mechanisms for a decreased discharge at the crisis.

The $\frac{systole}{cycle}$ *ratio during progressive anoxemia.* In a critical analysis of eight experiments, it was found that the duration of total systole shortened as the degree of anoxemia increased. This could, of course, be accounted for in one of four possible ways: 1. It might be caused by a fall in venous pressure, but as seen in figures 1 and 2 systole continued to shorten even when venous pressures rose to extreme heights during the post-critical periods. 2. It could be assigned to an increased aortic pressure but, again, systole shortened whether systolic pressure was high or low. 3. It might be a function of the increasing heart rate. 4. It could be due to a direct effect of anoxemia on the heart.

To differentiate between the latter two, the s/c ratios of several experiments were plotted in relation to a theoretical curve derived from a long cycle after the method described by Wiggers and Katz (1920). Figure 4 shows such a graph in which the circles represent data before the crisis and the crosses, cycle values after the crisis. It is apparent that, in the early stages of anoxemia, the points fall very close to the line but as the heart reaches 150 or more, all actual values are decidedly lower. A further analysis of the crisis and post-crisis changes indicates that, as the heart slows to a remarkable degree, there is no corresponding increase in the duration of systole. This is shown by the fact that the crosses in figure 4 fall far below the theoretical line. Experiments in which the vagi were cut and when the heart rate remained constant until just before the crisis showed no variation in duration of systole. As the heart accelerated near

the critical stage, systole decreased in length. As the rate subsequently slowed again there was a further decrease in systolic ejection quite contrary to expectation. As these results are similar to those obtained by stimulation of the accelerator nerves (Wiggers and Katz, 1920) it is impossible to state whether in these experiments this extra shortening of systole is due to a direct effect of anoxemia on the heart or to the excitation of the accelerator mechanisms.

It is interesting to note that this excessive decrease in duration of systole is perhaps related to a similar change observed by Feil and Katz (1923) on patients with decompensated hearts and explained by them as due to an overloading of the muscle. We suggest that the anoxemia which must have been present may account for their findings.

Changes in intraventricular pressure during progressive anoxemia. Having analyzed the changes taking place in the circulation during anoxemia and

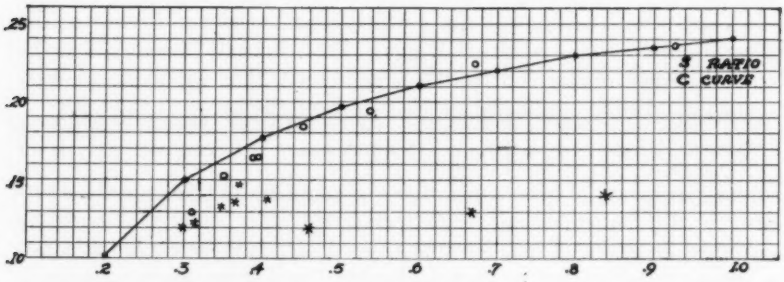

Fig. 4. Plot showing theoretical s/c ratio curve and actual values of systolic duration. Description in text.

having ascertained as far as can be done in naturally breathing animals the probable factors responsible for these changes, further experiments were carried out in which left intraventricular and aortic pressures were simultaneously recorded. Such experiments are valuable even though the opening of the chest and the introduction of mechanical means of lung inflation lowered the arterial pressures considerably below those found in naturally breathing animals and, as the degree of lung inflation was kept constant by the artificial pump, eliminated the compensatory increase in pulmonary ventilation which occurs in naturally breathing animals.

Curiously enough, it was found that in spite of these conditions such animals apparently tolerated anoxemia quite as well as those breathing naturally and with better arterial pressures at the start. The crisis appeared to occur when the oxygen percentage was somewhat lower than 7 or 8 per cent and death did not take place until the oxygen percentage had decreased to 5 per cent or less. Furthermore, the course of such

experiments (as shown by plots of changes in heart rate, arterial and venous pressures, systole and its phases) paralleled those of naturally breathing animals.

The detailed changes of intraventricular and aortic pressure curves at various stages of anoxemia are shown in the eight tracings of figure 5 which were selected from a large number of tracings taken during the course of an experiment. Segment I represents a normal control from an animal with cut vagus nerves. It was taken just before it had been connected to the respirometer and is almost identical with another record taken immediately after connection. The venous pressure was 45 mm. saline, systolic and diastolic pressures were calculated to be 65 and 36 mm. Hg respectively The heart rate was 158 per minute; systole averaged 0.2 second, systolic ejection 0.146 second, making the isometric contraction phase 0.054 second.

The first evidence of any circulatory change occurred in records taken 35 minutes later where the oxygen of the inspired air had reached 15.5 per cent. The changes were, however, comparatively slight and of the same character as those described later during the course, where they were more distinctly indicated.

Segment II is a record taken 49 minutes after rebreathing had begun and when the oxygen percentage had been reduced to 11.5 per cent. The venous pressure had slowly fallen until now it was 35 mm. saline; systolic and diastolic pressures had changed very little, systole now being 66 mm. and diastole 32 mm. Hg. The pulse pressure, as is shown directly on the records, increased slightly in spite of an acceleration of the heart to 168 per minute.

While such changes may conceivably be due to the combination of an increased heart rate and a lowering of peripheral resistance (as previously discussed), the contour changes of the curves, in this instance give no evidence of such a change. This, however, it must frankly be admitted, may be due to the relatively low arterial pressures existing in these experiments. Furthermore, all the factors which have a tendency to decrease the systolic discharge are present in moderate degree; the venous pressure is slowly falling, the heart cycle is shortened and the duration of systole and systolic ejection have decreased to 0.176 and 0.135 second respectively.

On the other hand, there is distinct evidence that compensatory mechanisms are being brought into play to neutralize such a tendency. The initial pressure in the left ventricle has increased in spite of falling venous pressures, the reason for this remaining undetermined. The duration of the isometric contraction phase has decreased from 0.054 to 0.043 second and the gradient of the isometric rise of intraventricular pressure has become steeper, thus confirming previous deductions that the velocity of ventricular contraction has increased. This and the fact that the steep-

ness of the aortic pressure curve increases may be taken to mean that expulsion of blood occurs more rapidly, for Wiggers and Katz (1920) have found such a correspondence. Whether these compensatory efforts are complete so that the volume of systolic ejection has now returned to or exceeds normal can not be stated; it is significant, however, that they are being set in operation relatively early in anoxemia.

Whether the larger pulse pressure at a higher heart rate is interpreted as due to increased systolic ejection, to a peripheral dilatation or to both, one fact remains certain, viz., that it can not be interpreted otherwise than as indicating an increased minute flow through the vessels of the body, and that therefore an improved circulation is a factor in compensating for the diminution in oxygen within the blood.

Segment III is a specimen of the records obtained 65 minutes after connection to the respirometer and when the oxygen percentage of the improved air had fallen to 9.5. The venous pressure was 40 mm. saline, systolic and diastolic pressures were 61 and 30 mm. respectively. These figures as well as the aortic tracings themselves show that the pulse pressure had decreased slightly from that of segment II, though still equal to the value of segment I. The heart rate remains unaltered. Systole is further reduced to 0.172 second but systolic ejection slightly increases to 0.140 second. The initial pressure is further increased but the gradient of the pressure rise is not quite so steep in the intraventricular pressure curve. There can be little doubt but that systolic discharge has decreased somewhat since segment II.

Segment IV shows a record taken 87 minutes after connection to the respirometer and the oxygen in the inspired air had now decreased to 6.3 per cent. The venous pressure was 55 mm. saline, systolic and diastolic pressures were 67 and 28 mm. respectively. The pulse pressure, as is obvious in the record, had increased definitely. The heart rate was still 168 per minute. Systole now had a value of 0.148 second and systolic ejection was reduced to 0.124 second, the isometric contraction phase decreasing.

Whatever doubt may have existed previously as to whether systolic discharge was increased, there can be no misinterpretation now either of the fact or of the mechanisms responsible for it. A mere glance at the record shows that the initial pressure has further increased, that a pronounced increase in the gradient of the isometric pressure rise occurs, that the pressure maximum is higher and that the velocity of systolic ejection is increased.

Obviously, anoxemia has two distinct effects on the cardiac mechanisms which are of an opposing nature as far as systolic discharge is concerned; the abbreviation of the interval of systolic discharge acting to reduce it; the increased velocity of contraction tending to increase it. During the

earlier stages, there is doubt as to which predominates but, as anoxemia becomes more pronounced, the latter factor over-compensates for the shortened systolic ejection phase. Such a reaction is not unknown in physiology; it is, as Wiggers and Katz (1920 have shown, the precise reaction by which the heart delivers larger systolic volumes when stimulated by epinephrin, and is also the way in which it acts in reaction to the stimulation of the accelerator nerves. As the accelerator nerves are intact in these experiments it is consequently impossible to state whether the reactions of anoxemia are due to a direct effect on the heart or whether they are inaugurated by way of the accelerator mechanism, nor is it possible to state whether they are the direct effects of anoxemia or result from the increased concentration of "metabolites" or adrenalin in the circulation.

Segment V shows records taken 98 minutes after the beginning of rebreathing. The oxygen had decreased to 5.8 per cent. The heart rate was 167 and the venous pressure had increased to 85 mm. saline. The systolic pressure had decreased to 64 and diastolic to 37 mm. Hg, the pulse pressure being reduced. This set of events has previously been interpreted as indicating the very beginning of circulatory failure at the crisis. The reduced pulse pressure together with the lowered systolic pressure maximum both in the aorta and ventricle clearly indicate that circulatory failure is initiated by a reduction in the volume of the systolic discharge, though pulse pressure values alone may still be above the normal shown in segment 1.

A further study of the tracings shows that this in turn is due to the fact that the continued abbreviation of systolic ejection more than counterbalances the increased velocity of contraction. While the initial tension and gradient of the contraction curve increase still further, the phases of systole and systolic ejection are so greatly reduced that this is just beginning to dominate the volume of systolic discharge. Actually systole is now reduced to 0.129 second and systolic ejection to 0.112 second.

While such effects are distinct danger signals, it should be recognized nevertheless that a perfectly adequate circulation is still being carried on and could probably continue to be carried on if the oxygen percentage were not further decreased.

The real change of significance in circulatory failure soon appears, however. Segment 6 shows a record taken 8 minutes later when the oxygen percentage in the inspired air had further lowered to 5.5. The venous pressure had risen to 102 m. saline, the heart rate was 158, systolic and diastolic pressures were 56 and 37 mm. Hg respectively, the pulse pressure decreasing. A careful study of these records shows that the real failure inaugurated at this time is not occasioned, as previous evidence had forecasted, by a further abbreviation of systole and systolic ejection but by the fact that the increasing velocity of cardiac contraction, evi-

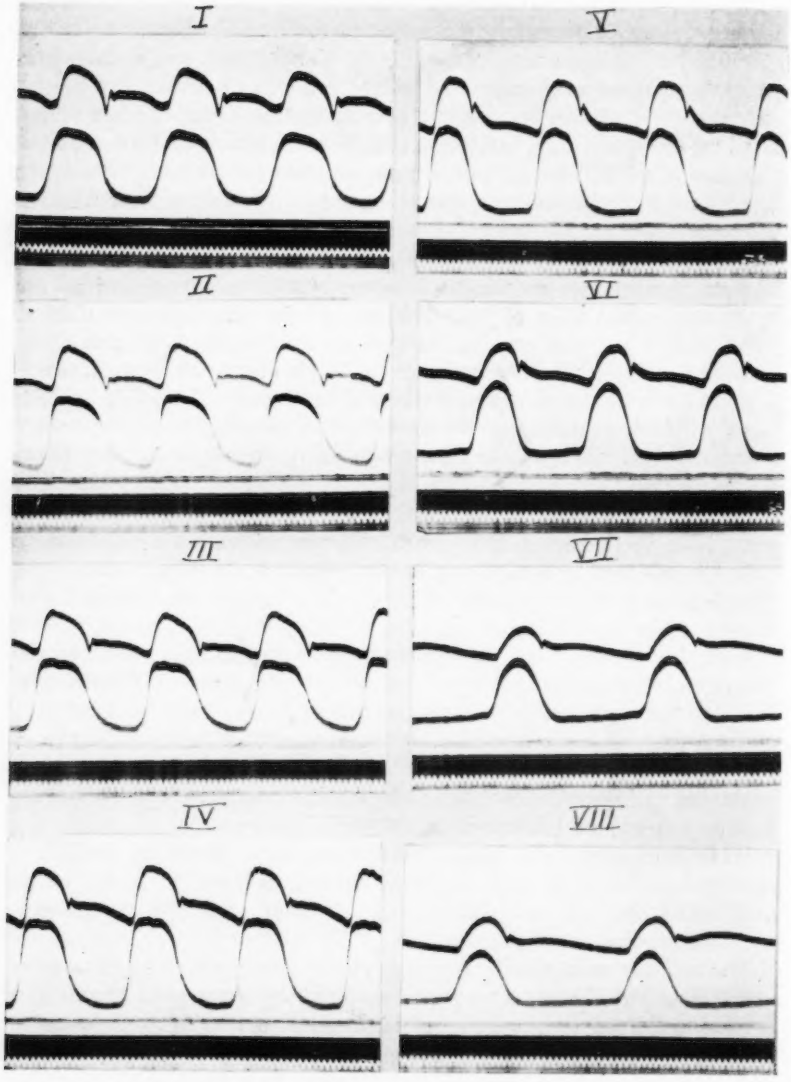

Fig. 5. Eight segments of records showing changes in aortic and left intraventricular pressures during progressive anoxemia. Artificial lung inflation, vagi cut. Segments I to IV before crisis; V, at crisis; VI to VIII after crisis. Further description in text.

denced by the steeper pressure gradient, is now converted into a contraction of reduced velocity as evidenced by the declining gradient of the pressure rise. As soon as the compensating mechanism which heretofore more or less counterbalanced the tendency of a reduced ejection phase to abbreviate systole is abolished, or rather converted into a mechanism assisting

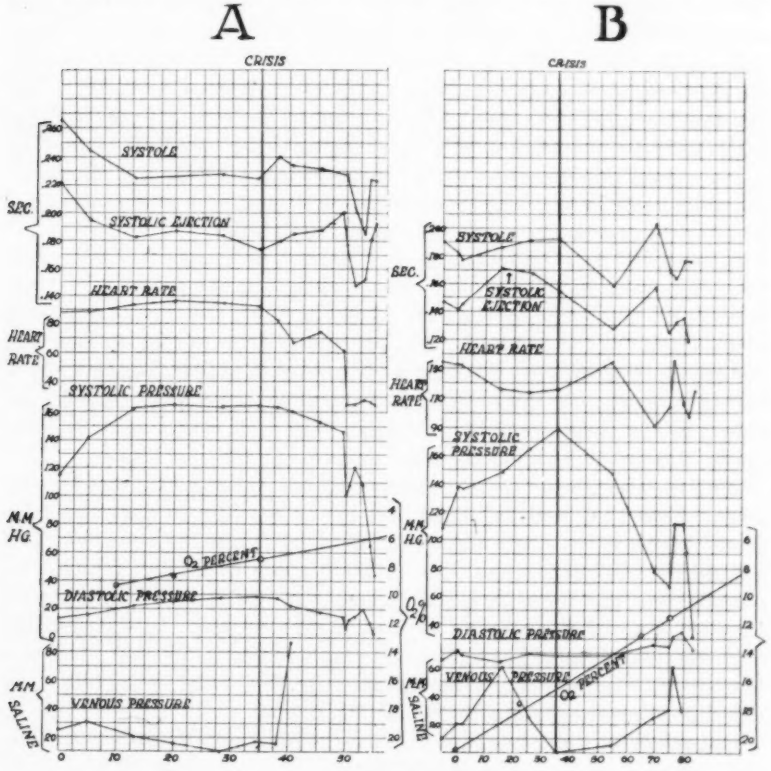

Fig. 6. Two charts showing data during anoxemia in dogs with distemper. A, mild infection; B, right-sided pneumonia. Description in text.

in the production of a reduced discharge, circulatory failure develops rapidly and progressively.

From this time on the changes are merely of degree, as is seen in segments VII and VIII of figure 5. The venous pressure rises progressively reaching 135 mm. of saline in the last record shown; the heart rate now decreases progressively and rapidly, being 98 per minute in the last record. Systolic and diastolic pressures fall. As the heart slows the

duration of systole and systolic ejection increases again slightly but never to levels anticipated. Thus in the last record the duration of systole and systolic ejection are 0.135 and 0.122 second respectively, i.e., far shorter than at a heart rate of 158 at which the experiment started (cf. segment I).

The changes of initial tension are interesting in the last records. At first it continues to increase still further as shown in segment VII but later progressively decreases as shown in segment VIII. This may throw some light on the cardiac dilatation which is shown by inspection to increase gradually up to the crisis but which rapidly progresses thereafter. The fact that the initial pressure increases up to the last segment suggests that until this time dilatation is of a physiological order being determined by the increased initial tension within the ventricle. At the end, however, the lack of relationship suggests that a pathological type of dilatation due to an active decrease in ventricular tonus, supervenes.

Influence of acute infections on the circulatory reactions to anoxemia. The impression, in tests on man, seems to be that the presence of acute infections, even though of relatively minor character, reduces the resistance to anoxemia (Whitney, 1918). An opportunity arose during the course of our experiments to study the reaction to anoxemia in four dogs severely ill with distemper. In three cases necropsy showed no gross pathological lesions of the lungs while in the fourth dog, there was a massive lobar pneumonia involving all of the right lung except the upper lobe.

With the exception of the dog with pneumonia, the results of these experiments showed no essential differences in the reaction to anoxemia from those obtained on healthy dogs. The detailed results of two experiments are plotted in figure 6, plot A being from a dog with lungs uninvolved, plot B, from the dog with severe pneumonia. In three of these experiments, we noted the absence of cardiac acceleration. In one instance only was there a rise of heart rate comparable to that in figure 1. One sees that the rate actually fell in one instance (fig. 6, B), and in the other two it remained practically unaltered (e.g., fig. 6, A).

Excepting the dog having pneumonia, the resistance to low oxygen tension was as great as in the normal dogs. Death occurred after about $1\frac{1}{2}$ hours, as in the normal dogs, and the crisis occurred when the oxygen of the inspired air had dropped to $6\frac{1}{2}$ or $7\frac{1}{2}$ per cent.

One dog developed a definite heart block, when the oxygen was 15 per cent, making it difficult to be quite sure when the crisis occurred. In the dog having pneumonia, however, the crisis began when the oxygen in the inspired air was still 16 per cent. Obviously, extensive consolidation of the lung is an extremely unfavorable complication in exposure to low oxygen tension.

SUMMARY

The dynamic changes in the heart and circulation were studied in dogs during the course of a progressive decrease in the volume per cent of oxygen in the inspired air, analyses being made from time to time with the Haldane apparatus. Changes in effective venous pressure were followed and records of heart sounds or left intraventricular pressures were taken synchronously with pressure changes in the arterial system. From these records, data as to changes in heart rate, duration of systole, systolic ejection and isometric contraction phases, systolic and diastolic blood pressure were derived, plotted and studied. In addition, the detailed changes in the left ventricular pressure changes were analyzed.

The following interpretations as to the effects of progressive anoxemia on the heart and circulation are formulated:

1. During the progressive increase in the degree of anoxemia previous to the circulatory failure at the crisis, which generally occurs when the oxygen percentage has been reduced to about 9 per cent, there are definite indications that the circulation improves and thus helps to supply the tissues with normal volumes of oxygen. When the vagi are intact and vagal tone is good, the heart accelerates progressively, partly on account of a diminution of vagal tone, but, to a lesser degree, to an accelerator stimulation or a direct cardiac action. The systolic pressure tends to increase and the diastolic pressure either remains unaltered or slightly increases, thus tending to increase the pulse pressure. Evidence is presented that, especially in the earlier stages, this may be due to the combination of cardiac acceleration and a reduced peripheral resistance but as anoxemia becomes even moderately severe or when no increase in heart rate occurs it is certainly due to an augmented systolic discharge. The detailed cardiac mechanisms through which this is accomplished are analyzed. It is shown that anoxemia has two opposing effects on the heart, the balance of which determines the systolic discharge. In the first place, it reduces the effective venous pressure, thereby diminishing the fundamental filling pressure and it abbreviates greatly the phase of systolic ejection thereby reducing the time interval for ejection. In the second place, it raises the initial tension in the left ventricle, and increases the velocity of ventricular contraction (as gauged by the gradient of the pressure rise and abbreviation of the isometric contraction phase) thereby increasing the rate of ventricular ejection. During the course of a progressive anoxemia, these two factors remain evenly balanced during the early stages and tend to maintain the systolic discharge normal or to increase it slightly, then, a period may be inserted where the first factor dominates and the systolic discharge decreases; but, as the demand for oxygen becomes more intense, the latter factor *always* dominates and increases the systolic discharge. Increased

systolic discharge, an increased rate of the heart and a reduced peripheral resistance all combine during anoxemia to increase the minute flow of blood through the body.

2. The circulatory crisis in anoxemia occurs when the oxygen supply is diminished to a point where the hitherto beneficial influence on the heart rapidly changes to a deleterious one. The first evidences of such failure are found in the decline of systolic and diastolic pressures and a reduction of the pulse pressure. As the venous pressure rises rapidly at this time and the arterial pressure curves show no change in contour indicative of an alteration in the peripheral circulation, the sudden failure of the circulation is not of a shock-like nature but is distinctly due to a reduction in minute output of the heart. This cardiac failure definitely precedes the final slowing of the heart; it also begins before the final cessation of respiration in naturally breathing animals and occurs quite as effectively when the lung inflation is maintained by artificial means.

Evidence is presented to show that this is due to a reduced systolic discharge. This begins when the velocity of contraction is still great but when the duration of systolic ejection has been so far reduced that it dominates the volume ejected during each systole. During the stage, interpreted as the beginning of the crisis, a very effective circulation is still maintained, however, in fact evidence indicates that the minute discharge is still greater than under normal conditions.

The real catastrophe occurs several minutes later when the gradient of the intraventricular pressure curve and the lengthening isometric periods show that now the velocity of ventricular contraction is no longer increased beyond the normal but, on the contrary, is decreased. The combination of the slow speed of contraction and the continuing abbreviation of the ejection phase determine the rapid decrease in systolic ejection and the early decline of arterial pressures.

Soon the great slowing of the heart is added as a factor and this is the more powerful in promoting the rapid diminution of minute discharge because as the cycles lengthen the duration of systolic ejection continues far below the natural value and together with the depressed contraction cause the delivery of systolic volumes which are not even approximately normal at much greater rates.

3. The evidence is incomplete as to the exact mechanisms affected by oxygen lack. Attention is called to the fact that the pronounced cardiac acceleration is absent when the vagi are cut but as some acceleration still persists in many experiments, the possibility that the accelerator mechanisms is stimulated must be borne in mind. Attention is further called to the fact that the excessive abbreviation of systole, the great increase in the gradient of the ventricular contraction and the increasing systolic discharge produced by anoxemia are similar to the effects of small doses

of adrenalin or of stimulating the accelerator nerves. It is obvious, therefore, that up to the crisis it is unnecessary to assume any direct effect of anoxemia on the heart for all the stimulating effects of anoxemia can be accounted for by its effect on the vagus and accelerator mechanisms. That similar effects might also be produced from a direct action on the heart can of course not be disproven. The depression of the cardiac contraction shortly after the crisis which occurs in spite of a rapidly increasing initial tension, is not characteristic of any known nervous reaction on the heart and is soon followed by disturbances in rate and rhythm so as to leave no doubt that it is due to a direct effect of the oxygen deficiency on the heart.

4. Acute infections, such as distemper, do not appear to modify the course of events during anoxemia, except that the characteristic acceleration of the heart may fail to occur even when the vagi are intact. Consolidation of the lungs found in one animal had the effect of hastening the crisis, which then occurred at relatively high percentage of oxygen in the inspired air.

In conclusion we wish to thank Prof. Carl J. Wiggers for his assistance in planning this research and in interpreting the results.

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FACTORS INFLUENCING THE EXCRETION OF UREA

II. Diuresis and Caffeine

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Several investigators have demonstrated the fact that under standard conditions renal urea output, expressed in terms of the ratio $\frac{\text{Urine urea per unit time}}{\text{Blood urea concentration}}$, is constant within relatively small limits of variation when large volumes of urine of quite different magnitudes are being excreted. In other words, the rate of excretion of urea is independent of the rate of excretion of water during periods of very active diuresis.

It is still a question whether or not there is some relation between urine volume and the rate of output of urea when smaller volumes of urine are being excreted. Austin, Stillman and Van Slyke (1921) conclude that the rate of urea output increases "approximarely (a) in simple direct proportion to the blood urea concentration, and (b) in proportion to the square root of the rate of volume output of urine per unit body weight as long as the volume remains within ordinary limits." Addis and Drury (1923) state that there is no relation between volume and urea output though they grant that less urea is excreted when urine volumes are very low than when very high.

The data from which Addis and Drury draw their conclusions were derived from samples of urine taken under specified conditions but without reference to the condition of activity of the kidney in periods preceding and succeeding their collection. Austin, Stillman and Van Slyke made their collections during the first portion of the periods of diuresis induced by drinking water. It is believed that the present work has reconciled these two conflicting views by a study of the relation between the urea and the volume output throughout entire cycles of diuresis beginning in a control period and ending with the return of volume to the initial level.

The disputed problem of the influence of caffeine on urea excretion has been approached from the same angle by determining the effect of caffeine injected at various points in the period of diuresis.

METHODS. The greater number of the following experiments were performed on two healthy dogs with denervated kidneys. For a lesser number four other dogs, which had been operated in a similar manner, and two unoperated dogs, which served as controls, were used.

Urine was obtained by catheterization, the catheter being left in place during the entire experimental period. The urine obtained during the first thirty to forty minutes after catheterization was discarded. It was collected thereafter at fifteen minute intervals into graduated cylinders, the catheter being carefully moved about in the bladder at the end of each period until it seemed emptied. The catheter was then squeezed free of urine as long as great a portion of its length as possible. By this procedure the output of the kidneys was obtained as accurately as seemed feasible under the conditions of the experiment.

Each sample of urine was centrifuged unless perfectly clear, and analyzed for ammonium by direct Nesslerization and for urea by the method of Folin and Youngburg (1919) using the Koch-McMeekin modification of Nessler's solution. Since the ammonium nitrogen represents a relatively small amount of the total nitrogen determined by this method, it was not subtracted, the so-called urea nitrogen being in reality urea plus ammonium nitrogen. Depression of freezing point determinations were also made on selected samples.

Blood drawn from the external saphenous vein into a dry syringe containing a few crystals of potassium oxalate was analyzed for urea nitrogen by the method of Folin and Wu (1919) using a modification suggested by Dr. F. C. Koch.

In order to obtain a condition as uniform as possible to serve as a standard for comparison, the dogs were kept for twelve hours without food or water before each experiment and urine was collected for a control period averaging about an hour in length before diuresis was produced. Water and isotonic saline administered by stomach tube, or intravenous injections of 40 per cent sodium sulphate solutions, were used for this purpose.

RESULTS AND DISCUSSION. *1. Diuresis.* Forty experiments giving data on animals in the control condition and involving periods of from one-half to five hours duration show that for any given concentration of blood urea the concentration of urine urea remains virtually constant through the range of volumes averaging from 124 cc. to 220 cc. per 24 hours. Within these average limits, therefore, the total urea output and the $\frac{\text{Total urine urea nitrogen}}{\text{Blood urea nitrogen}}$, $\frac{D}{B}$ ratio, vary directly with the volume. A depression of water output below the average level of 124 cc. is accompanied or immediately followed by a compensatory increase in urine urea concentration. Periods of diuresis induced either by water given orally or by the intravenous injection of a hypertonic solution of sodium sulphate

produce a sequence of events illustrated by figure 1 and the first portion of figure 2.

Increase in volume over the average amount of 220 cc. per 24 hours is accompanied by a decrease in concentration of urea which, however, proceeds at a definitely slower rate than the increase in volume for periods of from 15 to 105 minutes. A marked increase in total urea output and in the $\frac{D}{B}$ ratio which lasts, on the average for 45 minutes, is produced by this lag in readjustment. The increase bears no constant relation to volume but is determined rather by the rate of onset of diuresis and by the condition of the kidneys, which respond to increased excretion of water much more promptly on some days than on others under seemingly identical conditions.

The drop in the concentration of urea continues until the total urea excreted reaches a level termed for convenience the diuresis level, at which it remains, within the periods covered by these experiments, as long as active diuresis continues unless blood urea diminishes. In that case urine urea falls a corresponding degree, the $\frac{D}{B}$ ratio remaining constant. In only two experiments out of 32 involving periods of continued diuresis was the $\frac{D}{B}$ ratio depressed below the values obtained in the control period. In the remaining 30 it was well above the ratios obtained for the lower volume of urine of the control period and essentially the same as that obtained for volumes averaging around 220 cc. per 24 hours.

Apparently the water output yielding optimum conditions for the excretion of urea in the dog under the conditions of these experiments is rather low and greater diuresis does not accelerate urea excretion after the initial period of readjustment.

The cessation of diuresis is accompanied by a lag in the compensatory change in concentration of urine urea of the same type as that occurring at the onset of diuresis with a resulting depression of the $\frac{D}{B}$ ratio of variable duration. The extent of the depression depends on the height of the previous diuresis, the rate of drop and the reactivity of the kidneys. The combination of conditions which results in the greatest and most enduring depression is previous high diuresis with its correspondingly low urea concentration, a rapid return to normal volume, and unreactive kidneys.

Usually the $\frac{D}{B}$ ratio returns to normal promptly following a brief period of diuresis though strictly speaking the kidneys often return to full activity more slowly than does the $\frac{D}{B}$ ratio, judging from the fact that for some

time they require a larger volume for the excretion of that amount of urea than during the control period. On the other hand, the recovery tendency is in some cases so strong as to persist into the first period of the next diuresis (fig. 1).

After a more prolonged diuresis the return to normal may be as active as after brief diuresis but frequently the return is very slow or but partial. In the latter type of reaction the concentration rises rapidly toward normal, then ceases, the kidneys being unresponsive to further decrease in urine volume for some time with the result that urea output becomes proportional to volume output and greatly depressed for lower urine volumes.

The ammonium output follows essentially the same curve as does the urea output (fig. 1) but the depression of freezing point falls more rapidly and recovers much more slowly (table 1). Determinations of chloride, which were made in a few experiments, indicate that they are in large measure responsible for this for the concentration of chlorides recovered at a much slower rate than either the urea or the ammonium. This same contrast between the behavior of chlorides and urea occurs in experiments on rabbits reported by Morris and Rees (1923), the behavior of uric acid corresponding to that of urea.

In view of the slow readjustment occurring at the onset of diuresis and of the unresponsive condition of the kidneys, which at times follows prolonged periods of diuresis accompanied by high water output, the low $\frac{D}{B}$ ratio, low ammonium excretion, and depression of freezing point accompanying the termination of any diuresis cannot be regarded as a compensatory reaction. Rather it would seem evident that the kidney recovers more or less slowly from the upsets in its functioning which volume changes involve, the lag in response varying somewhat for different constituents of the urine. The end result may or may not be compensatory.

This phenomenon is more easily explained by the secretion theory than by the theory of filtration and reabsorption. Furthermore, the fact that urea, ammonium, uric acid, and changes in the depression of freezing point have followed essentially the same curve in these experiments indicates that the same type of function is concerned in the excretion of all urine solids. If this reasoning be correct, the evidence presented by Nash and Benedict (1921) and by Stieglitz (1924), that the cells of the renal epithelium are active in secreting acids and bases, makes secretion the mechanism concerned.

It is believed that many discrepancies in the literature concerning the relation of volume and of diuresis to urea output will be reconciled if the entire cycle of changes involved in any diuresis is taken into consideration and if the results are expressed in terms of the $\frac{D}{B}$ ratio. The same is true

for ammonium excretion under the conditions of these experiments and is doubtless true for many of the other constituents of the urine.

Concerning the relation between urine volume and urea excretion, inspection shows that, if only the earlier phases of diuresis are taken for statistics the conclusions of Austin, Stillman and Van Slyke (1921) that the rate of urea output increases "approximately (*a*) in simple direct proportion to blood urea concentration, and (*b*) in proportion to the square root of the rates of volume output of urine per unit body weight as long as the rate

TABLE 1

The effect of diuresis on the depression of freezing point and urea nitrogen output in grams per 24 hours

DOG	EXPERIMENT		ONSET OF DIURESIS	POST-DIURETIC PERIOD	
1	July 18th:	Volume, cc.	345.6	340.4	345.0
	1st diuresis	Δ	3.085	1.80	1.80
		Urea N, grams	5.727	2.783	5.075
	2nd diuresis	Volume, cc.	1084.8	672.0	1050.0
		Δ	1.12	0.89	0.77
		Urea N, grams	7.696	3.417	3.75
2	January 22	Volume, cc.	355.0	345.6	211.2
		Δ	4.825	1.605	2.74
		Urea N, grams	14.83	4.312	3.948
2	December 4	Volume, cc.	864.0	240.0	846.0
		Δ	1.84	0.45	0.35
		Urea N, grams	9.8	0.912	4.48
2	February 3	Volume, cc.	297.6	221.0	
		Δ	4.04	2.51	
		Urea N, grams	10.35	4.625	
1	August 4	Volume, cc.	638.4	768.0	297.6
		Δ	1.08	0.38	1.31
		Urea N, grams	8.166	1.536	1.878

remains within ordinary limits," would doubtless hold true. If, on the other hand, periods taken at random are selected as the basis for comparison, or if the diuresis level has already been established before the experiment begins, the conclusions drawn by Addis and Drury (1923) and by one of us (Bourquin, 1924) that there is no direct relation between volume output and rate of urea secretion are verified (table 1).

The effect of a period of diuresis on total ammonium and urea output will vary. For the cycle including the onset of diuresis until the $\frac{D}{B}$ ratio

becomes normal, the result may be an increase, no change, or a decrease if expressed in terms of the averge grams of urea and of ammonium for the period per unit time. A single prolonged diuresis will result in a marked increase in output if the recovery is prompt and if the blood urea concen-

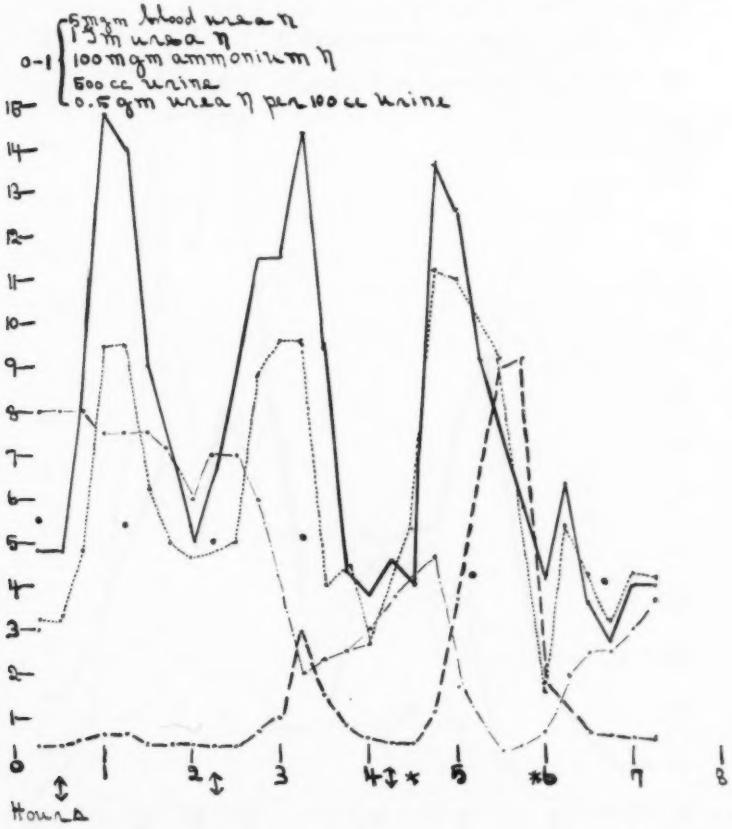

Fig. 1. The effect of diuresis on the excretion of urea and ammonium. Urea nitrogen in grams per 24 hours, ————; ammonium nitrogen in milligrans per 24 hours, - - - - - - - -; urea nitrogen in grams per 100 cc., —·—·—·—·; volume of urine in cubic centimeters per 24 hours, —·—·—·—·; 250 cc. of water orally, ↕; 1 mgm. caffeine per kilo intravenously,*; • blood urea N.

centration does not diminish. Otherwise the end result may be an actual decrease in total output. Characteristically the result of repeated diureses of equal intensity, the one immediately succeeding the other, as a diminution in output in the second, and equal or decreasing amounts in the follow-

ing periods, while succeeding diureses of increasing intensity, result in equivalent amounts from period to period. The explanation for this

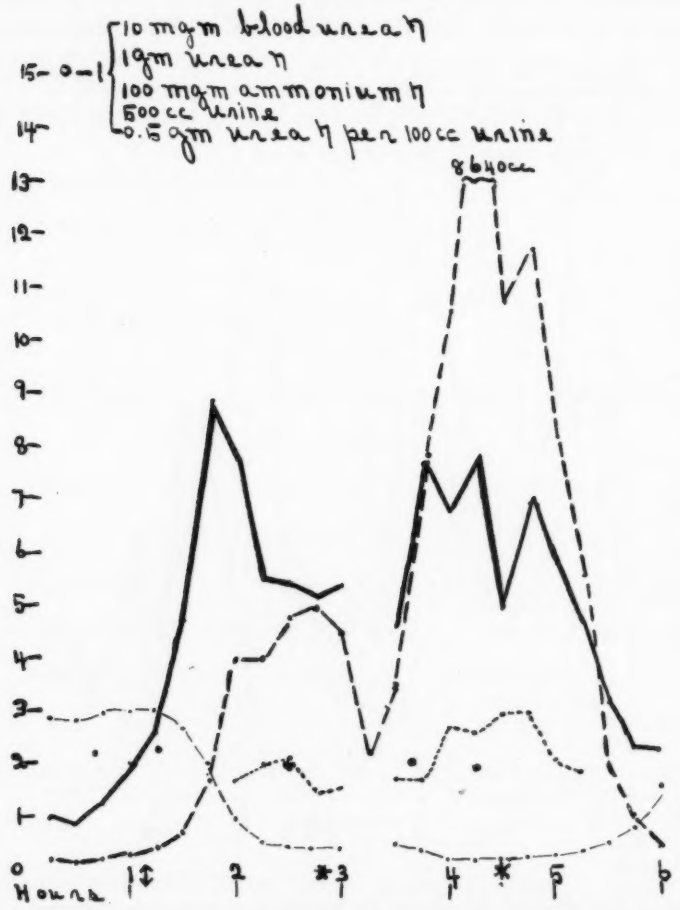

Fig. 2. The effect of caffeine injected after the diuresis level is established on diuresis and urea output. Urea nitrogen in grams per 24 hours, ————; ammonium nitrogen in milligrams per 24 hours, ----------; urea nitrogen in grams per 100 cc., —·—·—·—; volume of urine in cubic centimeters per 24 hours, —·—·—·—·—; 500 cc. of water orally, ↕; 1 mgm. caffeine per kilo intravenously, *; • blood urea N.

difference lies in the fact that if imperfect recovery in concentration takes place between periods of diuresis, the initial high urea output is missed unless the initial rise in diuresis is correspondingly greater (fig. 1).

Since periods of intense urea excretion at the onset of diuresis frequently seems to lower blood urea concentration the only true expression of urea output is the $\frac{D}{B}$ ratio. If the duration of the period be considered to

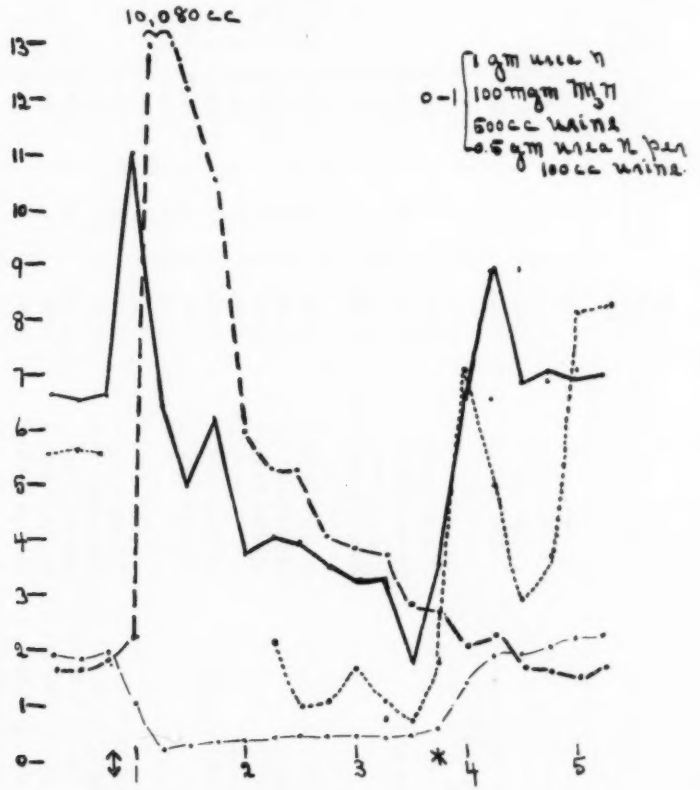

Fig. 3. The effect of caffeine injected during the period of the cessation of diuresis on the output of urea and ammonium. Urea nitrogen in grams per 24 hours, ————; ammonium nitrogen in milligrams per 24 hours, ---------; urea nitrogen in grams per 100 cc., —· —· —· —·; volume of urine in cubic centimeters per 24 hours, — ·—· —· —·; 40 per cent Na_2SO_4 solution intravenously. 1 mgm. caffeine per kilo intravenously, *.

include the output from the onset of diuresis until the $\frac{D}{B}$ ratio is normal, regardless of volume relations and subsequent events, and if the effect of diuresis is measured in terms of $\frac{\text{The average urea per unit time}}{\text{The average blood urea for the period}}$

TABLE 2

Experiments typical of the effects of diuresis on urea and ammonium excretion

EXPERIMENT	PROCEDURE	PHASE OF DIURESIS PERIOD	AVERAGE UREA NITROGEN PER 24 HOURS	AVERAGE NUMBER OF PERIODS	AVERAGE VOLUME PER 24 HOURS	AVERAGE AMMONIUM NITROGEN PER 24 HOURS	AVERAGE URINE UREA NITROGEN: AVERAGE BLOOD UREA N
			grams		*cc.*	*grams*	
Dog 2, January 8		Control period	3.652	7	136.0		22.8
		Control period	4.095	7	157.0		24.6
	Caffeine intravenously, 0.5 mgm. per kilo	Control period	3.923	6	163.0		23.8
Dog 1, February 3		Control period	2.77	4	214.0		12.59
	Water 500 cc.	Entire period	3.13	13	1,123.0		14.7
		Diuresis level	3.015	4	3,000.0		14.3
		Return level	3.159	3	409.0		13.9
Dog 3, August 9		Control period	6.947	8	180.0	0.3266	31.7
	Water 400 cc.	Entire period	7.505	10	2,320.0	0.3627	52.48
		Diuresis level	7.585	6	3,080.0	0.505	53.04
	New normal		2.248	2	681.0	0.2145	16.9
	0.9 per cent NaCl, 400 cc.	Entire period	5.94	10	1,742.0	0.331	50.3
		Diuresis level	7.49	2	5,700.0	0.415	57.3
	New normal		2.781	2	350.0	0.278	27.81
Dog 2, December 4		Control period	2.144	7	147.0		13.7
	Water 300 cc.	Entire period	2.80	16	2,400.0		16.66
		Diuresis level	3.374	4	3,630.0		18.5
		Recovery period	1.40	7	196.0		8.65
	Water, 300 cc. Caffeine 0.5 mgm. per kilo intravenously in sixth period.	Diuresis level	3.44	8	611.0		16.38

Dog 2, May 27		Control period	2.622	5	126.0	0.225	14.2
	Water, 700 cc. Caffeine 0.9 mgm. per kilo intravenously, eighth period of post diuretic recovery	Entire period	3.611	27	2,688.0	0.226	21.4
		Diuresis level	5.625	3	4,000.0	0.287	31.6
		Recovery period:					
		a, pre-caffeine	2.055	8	270.3	0.187	11.9
		b, post-caffeine	2.45	4	281.4	0.343	14.4
Dog 2, January 22		Control period	5.75	2	144.0	0.555	20.1
	Water, 250 cc.	Entire period	8.835	8	250.0	0.654	33.3
		Post diuretic return	6.32	2	180.0	0.555	26.08
	Water, 250 cc.	Entire period	7.92	8	422.0	0.666	32.77
		Post diuretic return	4.72	4	259.0	0.417	20.04
	Water, 250 cc.	Entire period	6.97	11	1,600.0	0.6227	33.19
		Diuresis level	7.019	2	4,407.0	0.732	32.19
		Post diuretic return	4.024	2	230.0	0.408	20.2
Dog 2, November 21		Control period	4.2	2	150.0		35.2
	Water, 250 cc.	Entire period	6.24	13	1,900.0		51.5
	Caffeine intravenously per kilo, 0.5 mgm. period 5, 0.4 mgm. period 9	Diuresis level	5.78	7	2,069.0		47.4
		Pre-caffeine	3.92	2	1,449.0		32.1
		Post-caffeine	6.87	5	3,050.0		57.25
	New normal, 1 hour later		1.28	6	238.0		11.03
	0.9 per cent NaCl 250 cc.						
	Caffeine 0.5 mgm. per kilo	Diuresis level	0.922	2	1,845.0		17.66

(that is, in terms of the average $\frac{D}{B}$ ratio for that diuresis) the results of 22 experiments show the average $\frac{D}{B}$ ratio to be nearly equal to the $\frac{D}{B}$ ratio of the diuresis level, of 4 to be less, and of 2 to be higher unless the result of the subsequent unreactive condition of the kidney be taken into consideration when it becomes less (table 2). In other words, the maximum effect which any period of diuresis produces has been shown to be an average output equivalent to the $\frac{D}{B}$ ratio for the diuresis level and in many cases the end result is a lesser average output.

2. Caffeine. The effects of caffeine were studied in the two dogs with denervated kidneys which were used for the major part of this work, and on the two unoperated controls. It was found, in accordance with numerous statements in the literature, that caffeine could be injected into the unoperated dogs during periods of active diuresis without inhibiting the excretion of water, and, as would be expected, could be injected into the animals with the denervated kidneys under any conditions without depressing the volume output.

The influence of caffeine on urea output might be purely incidental to the diuresis, which caffeine may cause, as suggested by several investigators; it might be due to changes in per minute volume flow through the kidney; to changes in blood composition; or it might be that of direct stimulation of the kidney to increased output of the various constituents of the urine over and above that produced by diuresis. If it acts in such a manner, the average urea output for the control and diuresis periods should be increased, the diuresis level should be higher and the return of concentration in the post-diuretic period should be more rapid. To test this caffeine was injected during the various phases of the diuresis period.

The result of injections of caffeine during the control period were negative in 6 experiments (table 2, expt. 1).

In 14 of 19 experiments in which caffeine was injected during a period of active diuresis, the $\frac{D}{B}$ ratio of the diuresis level was temporarily slightly increased. This increase was produced by a transient inhibition of the drop in concentration occurring at the onset of diuresis in 2 experiments. In the others, in which it occurred after the drop in concentration was apparently complete, it was produced by a large increase in the volume output without the accompanying decrease in urine urea concentration or with even a slight increase in it (fig. 2). These same results were obtained by Addis and Drury (1923a) in man under similar conditions. Inspection

will show that the crucial experiments on dogs under Barbital anesthesia reported by one of us (Bourquin, 1924) belong in this group.[1]

In the majority of experiments in which the entire period was followed, the temporary augmentation in urea output produced by the caffeine did not raise the average $\frac{D}{B}$ ratio for the period above that of the diuresis level preceding the caffeine injection, the post diuretic depression accompanying the great drop in volume compensating for the increase even when recovery was active. Also the injection of caffeine at the onset of diuresis failed to increase the average $\frac{D}{B}$ ratio for the period over and above that for preceding periods (table 2).

In 5 experiments not included in the above group, in which caffeine was injected during a period of low diuresis, when the kidney was in the unreactive condition as a result of a previous prolonged diuresis, caffeine produced no evident effects.

The kidneys are more sensitive to the effects of caffeine during the fall in volume output at the end of diuresis or immediately thereafter than at any other time. In 8 experiments the onset of any substantial degree of recovery in urea concentration was coincident with the injection of caffeine (fig. 3). In 8 of 14 experiments, in which recovery was already active, a transient acceleration in the rate of recovery was produced, which not only led to an inhibition of the post-diuretic depression but, to an increase in total urea above that of the previous period. Subsequent to such stimulation the kidney usually remained very active but in some cases, in which the previous activity had been intense, the recovery proceeded no further than the initial caffeine rise during the time it was observed in spite of the return of volume to normal. In the remaining 6 experiments caffeine had no perceptible effect upon the rate of recovery or upon the total urea output.

In each case the effect on the ammonium output and on the depression of freezing point was similar to the effect on urea though it frequently differed in degree.

Aside from changes in urea output incidental to diuresis, the possible points of action of caffeine in producing these effects are, as stated, the cardiovascular system, the composition of the blood, and the renal epithelium. The first action is not included in these experiments.

It was not thought necessary to investigate the influence of caffeine on renal circulation in the course of the present work for the effects of caffeine were super-imposed upon conditions which would of themselves insure an

[1] Attention is called to the printer's errors in columns 4 and 5 of the last experiment in the table published with that paper.

active flow of blood through the kidney and the numerous investigations of the relationship between per minute volume circulation and diuresis have led to the conclusion that if renal blood flow is active, diuresis is quite independent of further increase in it. The most thorough recent work bearing specifically on the effect of caffeine on the changes in per minute volume flow in the kidney, which have come to our attention, is that of Cushny and Lambie (1921) using a modification of the Barcroft and Brodie technic. They find that in rabbits caffeine produces a transient increase of 15 to 22.5 per cent in renal blood flow beginning one minute after the injection and lasting for from 4 to 5 minutes, followed by a return to normal or below normal, and a diuresis which long outlasts the transient augmentation of blood flow. Other investigators, missing the initial increase, report that caffeine does not increase the per minute volume blood flow at all but that there is an increase in volume shown by the oncometer. The latter might well occur without increase in rate of flow due to a more patent glomerular system, as reported by Richards and Schmidt (1924) if the distal ends of the arterioles maintained their tone. However, in case the per minute volume flow in the circulation as a whole is depressed as in cardiac decompensation or by surgical procedure incident to many crucial experiments this statement would not hold, the greater per minute volume flow in the system as a whole leading to a similar increase in renal blood flow, other things being equal, and this of itself would influence secretion under those conditions.

That changes in composition of the blood are probably not responsible is indicated by the fact that all three of the factors investigated change in the same direction and that caffeine does not alter blood urea concentration. The discovery of Fleisher and Loeb (1910) that in rabbits an increased elimination of chlorides resulting in an actual drop in the sodium chloride content of the blood follows injection of caffeine at half-hour intervals is further evidence that it acts directly on the renal epithelium as a mild stimulant.

SUMMARY

1. Urea in terms of $\frac{\text{Urine urea nitrogen}}{\text{Blood urea nitrogen}}$ $\left(\frac{\text{D}}{\text{B}} \text{ ratio}\right)$, ammonium, and depression of freezing point have been determined in the urine of dogs throughout entire periods of diuresis with and without injection of caffeine in an effort to learn the relation between urine volume and the rate of excretion of urea and other solids and the effect of caffeine on the kidney.

2. Volume has no constant influence on the output of urea or other solids, the ratio $\frac{\text{urea output}}{\text{water output}}$ depending upon the previous state of activity of the kidneys as follows:

a. In dogs which have been without food or water for 12 hours, urine urea concentration remains constant and the $\frac{D}{B}$ ratio varies with the volume output between the rough average limits of 124 cc. to 220 cc. per 24 hours.

b. The onset of diuresis is accompanied by an exceedingly high $\frac{D}{B}$ ratio due to the fact that the drop in urine urea concentration lags behind the initial increase in urine volume.

c. If diuresis continues the $\frac{D}{B}$ ratio drops to a level which is usually well above that established for the lower volumes excreted in the control period and which is maintained throughout the subsequent active diuresis through wide range of volume.

d. As diuresis subsides, and during the post diuretic period, there is a more or less prolonged period of depressed $\frac{D}{B}$ ratio, which is caused by the fact that urine volume returns to the pre-diuretic level more promptly than the urine urea concentration returns to that level.

3. Since the changes in ammonium output and in the depression of freezing point were similar to the changes in urea output, we conclude that renal function is characterized by more or less of a lag in the readjustment of the concentration, at which its solids are secreted, to change in urine volume.

4. The rate at which the urea concentration recovers at the termination of a period of diuresis determines whether the output of urea and other solids for the period as a whole will be increased, decreased, or left unchanged by that diuresis.

5. Caffeine in doses of from 0.5 to 1.0 mgm. per kilo produced a slight transient increase in urea output over the preceding level if injected during a period of active diuresis or during the post-diuretic drop and recovery phases of diuresis in some cases. The changes in output of ammonium and in the depression of freezing point are similar to the changes in urea output under the conditions of these experiments.

We are indebted to Dr. A. J. Carlson and to Dr. F. C. Koch for the use of their laboratories and for their generous interest and helpful suggestions.

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THE AUTONOMIC RHYTHM OF TURTLE HEART STRIPS AS INFLUENCED BY THE REGIONAL GRADIENT AND VARIOUS CONDITIONS[1]

I. The Duration of the Initial Inhibition of the Cardiac Strips

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The heart of the turtle is a peculiarly useful object for the study of the fundamental properties of the cardiac muscle; especially for observing the comparative behavior of the different parts and levels of the heart, and the comparative reactions of the rhythmic and non-rhythmic muscle. The heart may be cut into strips which may be suspended in solutions and attached to levers, to record their contractions. In proper solutions, these strips contract as rhythmically as a heart in situ. By rather slight changes in the ratio of ions the rhythmicity can be suppressed, so that the heart muscle contracts vigorously, but only in response to extraneous stimuli. These phenomena have been studied in their gross features by Howell, Greene, Lingle, Martin and others. In this paper we shall show that the ion-ratios, as also other properties, vary with the region of the heart from which the strip was taken, in a definite sequence that corresponds to the "cardiac gradient," i.e., right sino-auricular region, left sino-auricular region, base of ventricle, body of ventricle, apex of ventricle, auricular tissue devoid of sinus. The phenomena are, however, also influenced by a number of conditions, such as the species of the turtle, the season of the year; the aeration of the solution; and the temperature of the solution. These must be properly observed or controlled, else the results become needlessly complicated.

Method of experimentation. Cumberland Terrapins, Chrysemys elegans, generally between 7 and 8 inches in length, were used in all experiments. The animals were killed by a blow on the head, and the heart immediately removed and cut into strips, according to the regions to be studied.

[1] A preliminary communication of some of these observations was made to the "Joint Medical Conference" at Hongkong, January, 1925, and published in The Journal of Biochemistry, Tokyo, 1925, v, 87.

Sinus-auricular preparations: The ventricle was removed by a cut just above the auriculo-ventricular junction. A second cut was then made through the interauricular junction, so as to separate the two auricles. Each auricle was then lifted and excised so as to include a fairly long stretch of the vein.

Sinus free auricles: The heart was lifted by the ventricle, and the globular sides of the auricles were excised, as indicated by the dotted lines in figure 1. This excludes both the sinus and the intra-auricular band which contains sinus-tissue (Gaskell).

Transverse strips of the ventricle: These were cut as shown in figure 2, so that the ventricle was divided into three rings of approximately equal height. The base did not include the auriculo-ventricular junction.

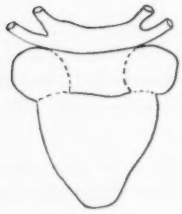

Fig. 1

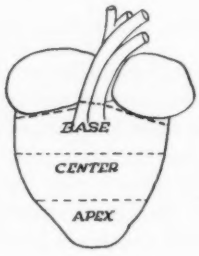

Fig. 2

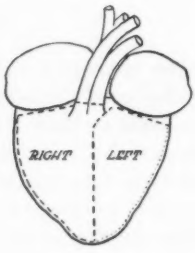

Fig. 3

Fig. 1. Posterior aspect of the turtle heart. The dotted lines show the cuts for the sinus-free auricles.

Fig. 2. Anterior aspect of the turtle heart, to show the cuts for the transverse strips.

Fig. 3. Anterior aspect of the turtle heart, to show the cuts for the longitudinal strips.

Longitudinal strips of the ventricle: The ventricle was cut away from the auricle below the auriculo-ventricular junction, and was then divided into a right and left half (fig. 3); and each of these again into an anterior and posterior half, so that four strips were formed. It may be premised that the properties of the longitudinal strips are determined mainly by the level at which the upper section is made; i.e., they may thus be given the rhythmicity of the base, center or apex. The right anterior strip contains the root of the aorta, which probably contains tissue of much higher rhythmicity.

The strips or segments were suspended as shown in figure 4, by means of fine copper wires and hooks, between a heart lever and a hollow L-shaped nickeled brass rod, which serves as a support for the muscles and as a delivery tube for the aeration of the solution. The fulcrum of the lever was

insulated from the stand by vulcanite washers so that a current sent through the wires must pass through the muscle. The wires were connected with the secondary coil of a Harvard inductorium, activated by a storage current of 2 to 4 volts, with the center of the secondary coil 8 cm. from the base of the primary coil. The suspended segment was immersed in about 30 cc. of the solution, contained in a conical graduate of about 50

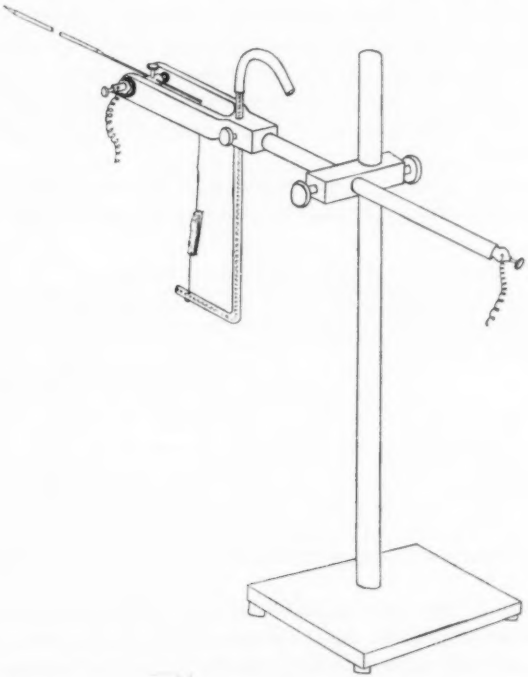

Fig. 4. Lever and support for heart-strips. The L-shaped support consists of a nickel-plated brass tube, which serves also for aeration. The connections of the strip are made of wire, but the supporting screws of the fulcrum are set in vulcanite (shown by the deep black rings). This forces the stimulating current to pass from the binding screws through the muscle.

cc. capacity. This graduate was set in a large water bath to regulate the temperature, which is very important.

The influence of aeration. When it is desired to maintain the heart in the best condition, a rapid current of air must be bubbled through the solution; but if it was expressly desired to place the heart under unfavorable conditions, the solution was left without aeration. Table 1 shows that aeration almost doubles the time during which the strip maintains a

spontaneous rhythm. The exhausted non-aerated hearts are irresponsive also to electric stimulation, and cannot be restored by aeration. If the aeration is stopped for short periods—10 to 15 minutes—the beats become weaker and often irregular, but may be restored perfectly by aeration. The benefits of aeration appear to be due solely to the supply of oxygen; for a lively stream of hydrogen does not improve at all over non-aeration (table 1).

The permissibility of copper-connections: Fearing a deleterious "oligodynamic" action of copper, all connections that came in contact with the muscle or solution were at first made of gold and platinum. Comparative experiments, shown in table 2, demonstrated that this refinement was superfluous, and copper wire and ordinary brass pins were used in all the later experiments.

Saline solutions: The cation-concentration of the turtle-serum is said to be represented by a Ringer's solution, containing NaCl 0.7 per cent, $CaCl_2$ (anhydrous) 0.026 per cent and KCl 0.03 per cent. This solution preserves the irritability of the heart (Greene, 1898), but is not optimal for rhythmicity. It does not interfere materially with the sinus, but may altogether suppress the spontaneous rhythm of the auricles, apex and base of the heart, an arrangement that is evidently favorable to the pacemaking supremacy of the sinus (Howell, 1898). Strips from all parts of the heart beat spontaneously, and for hours, in 0.7 per cent NaCl solution, so that this is a very convenient starting point for experiments. To this desired amounts of concentrated solutions of calcium chloride and potassium chloride may be added. The concentration of these solutions is always stated in terms of the anhydrous salts. The solutions were freshly made every 2 or 3 days, with glass-distilled water.

TABLE 1

Influence of aeration on duration of rhythm

Parallel longitudinal strips of the ventricle were suspended in a solution of NaCl 0.75; $CaCl_2$ (anhydrous) 0.025; glucose 1 per cent.

	DURATION OF RHYTHM (MINUTES BETWEEN FIRST AND LAST APPRECIABLE CONTRACTION)		
	Average	Range	Number of experiments
Aerated with oxygen	*178*	100–500	8
Aerated with hydrogen*	*110*	75–135	13
Non-aerated	*128*	80–170	6

* The hydrogen was prepared from HCl and Zn, and washed with NaOH solution.

DURATION OF PRE-RHYTHMIC INHIBITION. If a strip from a freshly-excised heart is immersed in a solution that is favorable for rhythm, a certain time elapses before spontaneous contractions become established.

This pre-rhythmic, or dormant or "latent" period, as it has often been called; or more properly, this "period of absolute inhibition," ranges from a few seconds to an hour or longer, according to conditions, and particularly according to the region of the heart from which the strip was taken. The existence of this inhibition is well known, but the conditions that influence it do not appear to have been thoroughly studied. It has been explained in various ways, but none of the explanations appear conclusively established, and they need not concern us at present. It will suffice to note that something or other is set in motion by the removal of the heart which inhibits or blocks or interferes with the rhythmicity; and that this inhibition diminishes and disappears with time. Assuming the cause of the inhibition to be essentially the same for all strips, the duration of the inhibition would vary inversely to the rhythmic tendency of the strip. This in turn depends essentially on *a*, the regional gradient of the heart; and *b*, the vigor of the preparation. This agrees fully with the data that we shall present.

TABLE 2

Influence of copper vs. gold and platinum connections

The arrangement was the same as in table 1.

	DURATION OF RHYTHM IN MINUTES					
	Gold and platinum			Copper		
	Average	Range	Number of experiments	Average	Range	Number of experiments
Oxygen	*194*	100–320	4	*240*	105–380	4
Hydrogen	*114*	85–135	6	*108*	75–130	5
Non-aerated	*125*	105–165	3	*133*	80–170	3

The period of absolute inhibition is usually succeeded by an intermediate period of partial inhibition, before the heart attains its full regular and maximal rhythm. This partial inhibition presents very interesting phenomena, which will be presented in some detail later. At this time it is important because its duration also varies with the rhythmic tendency, in the same direction as the absolute inhibition; so that it confirms and often extends the conclusions.

In the following data, the absolute inhibition includes the time from the immersion of the strips (immediately after the removal of the heart) to the first spontaneous beat. If no spontaneous beat had appeared in an hour, the heart was stimulated with a single make and break shock; and this was repeated every 5, 10 or 15 minutes, until a spontaneous rhythm started. In these cases the duration of the absolute inhibition is denoted as "$>$ 60 min." For the very rare instances in which repeated stimulation failed to evolke a continued rhythm, the duration of the absolute inhibition is denoted as "indefinite" (∞).

The *partial inhibition* may go through several stages, which may be grouped under the time necessary to attain *a*, regularity, and *b*, maximal rate. In certain types the heart does not become regular until it attains its maximal rate, so that the two points coincide. Table 4 illustrates these features in regard to the regional gradient and the temperature. We shall recur to this table after analyzing the data step by step.

The regional gradient of absolute inhibition: Table 3 shows that the duration of the absolute inhibition increases in the following order:

Sinus-auricle, Right
Sinus-auricle, Left
Ventricle, Base or Longitudinal
Ventricle, Center
Auricle, sinus free, Left
Ventricle, Apex
Auricle, sinus free, Right

This gradient reappears uniformly and dominates in all the cardiac phenomena that we have studied. It corresponds closely with the gradient of rhythmicity or pace-production that is usually accepted for the mammalian heart (Th. Lewis, 1921) and for the turtle heart (Meek and Eyster, 1923); except that the low rhythmicity of the sinus-free auricle is more definitely emphasized, and that the auricular-ventricular ring is not included in our data.

These gradients are checked very well by the comparison of strips taken from the same hearts. For instance, with ventricular strips, at 15°, the base starts before the apex in each of eight hearts; at 20°, the center starts before the apex in each of 5 hearts. At 20°, the left sinus-free auricle starts before the right in seven hearts; the right before the left in only one heart.

The longitudinal strips apparently take their rhythm from the base of the heart; the absolute inhibition is therefore the shorter, the nearer to the base the strip extends. The lower part of table 3 also shows that the right anterior quarter has a considerably shorter inhibition than the other quarters. This is doubtless due to the aortic bulb, which contains tissue of higher rhythmicity, in the right-anterior strips.

The gradient of partial inhibition: This also follows the same order (table 4) but brings out further significant differences. The right and left sino-auricular region both contract spontaneously as soon as they are immersed in NaCl solution; but whilst the more rhythmic right sinus region assumes the regular and maximal rate almost at once, the less rhythmic left sinus region requires a median of 5 minutes at 20° and of 44 minutes at 15°, to reach perfect rhythm. The differences between the ventricular base and apex are about equally marked in regard to the attain-

ment of the first spontaneous beat (at 20°, Quotient $\frac{\text{Apex}}{\text{Base}} = 7$), of the regular rhythm ($Q\frac{A}{B} = 9.4$) and of the maximal rhythm ($Q\frac{A}{B} = 7$).

The influence of aeration on the duration of absolute inhibition: Table 5 shows that non-aeration prolongs the total inhibition very markedly, by 1.3 times at 15°; by 2.7 times at 20°; and by three times at 30°. The deleterious effect therefore increases with the temperature as would be

TABLE 3

The regional gradient of absolute inhibition

Strips in aerated 0.7 per cent NaCl. Time in minutes between immersion and first spontaneous beat.

Region	20°C.			15°C.			$Q_{T}^{20°}$: $_{15°}$
	Median	Range	Number of experiments	Median	Range	Number of experiments	
Sinus-auricle Right	*0*	0–0	9	*0*	0–4	7	
Sinus-auricle, Left	*0*	0–9	9	*0*	0–7	7	
Ventricle, Base	*3*	0–17	10	*15.5*	0–29	7	0.2
Ventricle, Longitudinal	*3*	2–43	7	*16*	0–28	3	0.2
Ventricle, Center	*14*	8–>60	6	—	—	—	—
Ventricle, Apex	*21*	6–>60	21	*49.5*	21–∞	11	0.4
Auricle, sinus free, Left	*15.5*	3–60	8				
Auricle, sinus free, Right	*24*	5–60	8				
Auricle, sinus free, Left and Right	*18*	3–60	16				
Longitudinal strips in non-aerated NaCl							
Right Anterior (aortic region)	*5*	(0–23)	6	*10*	(0–21)	5	
Left Anterior	*6*	(3–16)	3	*19*	(14–28)	3	
Right Posterior	*8*	(6–12)	5	*21*	(12–38)	5	
Left Posterior	*8.5*	(5–32)	6	*21*	(14–21)	3	

expected since oxygen-deficiency delays the establishment of rhythmicity. This influence of oxygen on the arrested heart was rather unexpected; but Mr. R. L. Howard has found, in this laboratory, that the freshly excised heart muscle, when arrested by saccharose solution, has a fairly high metabolism (CO_2 production); and this, it may be added, also follows the regional gradient.

The influence of temperature on the duration of absolute inhibition: The data regarding this are contained in tables 3 and 5; they are reduced to temperature quotients in table 6. It is seen that raising the temperature

TABLE 4

The regional gradient of absolute and partial inhibition

Strips in aerated 0.7 per cent NaCl. Time in minutes between immersion and the phenomenon stated.

		FIRST SPONTANEOUS BEAT			REGULAR RHYTHM			MAXIMAL RHYTHM		
		M*	R	N	M	R	N	M	R	N
20°C.	Sinus auricle, Right	*0*	(0–0)	5	*0*	(0–0)	5	*0*	(0–0)	5
	Sinus auricle, Left	*0*	(0–0)	5	*5*	(0–9)	5	*5*	(0–12)	5
	Ventricle, Base	*3*	(0–5)	4	*10*	(2–13.5)	4	*15.7*	(8–30)	4
	Ventricle, Apex	*21*	(9–42)	4	*94*	(42–157)	4	*111*	(54–162)	4
Quotient $\frac{\text{Apex}}{\text{Base}}$		7			9.4			7		
15°C.	Sinus auricle, Right	*0*	(0–0)	4	*0*	(0–0)	4	*0*	(0–7)	4
	Sinus auricle, Left	*0*	(0–0)	3	*44*	(41–92)	3	*44*	(40–113)	3
	Ventricle, Base	*12*	(0–37)	4	*33*	(15–46.5)	4	*45*	(32–53)	4
	Ventricle, Apex	>60	(21–>60)	4	*>186*	(78–>264)	4	>186	(78–>264)	4

* In this and further tables the following abbreviations will be used to designate the numbers: "M" = median; "R" = range or extremes; "N" = Number of experiments entering into the median.

TABLE 5

The influence of aeration and of temperature on absolute inhibition

Longitudinal strips, time in minutes between immersion and first spontaneous beat, 0.7 per cent NaCl.

	TEMPERATURE											
	15°C.			20°C.			25°C.			30°C.		
	M	R	N	M	R	N	M	R	N	M	R	N
Aerated (Anterior and Posterior)	*16*	(0–28)	3	*3*	(2–43)	6	—	—	—	*1*	(0–6)	11
Non-aerated (Anterior and Posterior)	*21*	(6–∞)	19	*8*	(2–∞)	20	*9*	(3–>60)	18	*3*	(1–57)	7
Non-aerated Anterior	*20*	(6–>60)	10	*5.5*	(2–23)	8	*6.5*	(0–>60)	10			
Non-aerated Posterior	*21*	(12–59)	9	*8*	(6–>60)	12	*31*	(4–>60)	8			

generally shortens the absolute inhibition; but that the temperature quotient varies with several conditions.

The favorable effect of increasing temperature appears more pronounced:

a. For the base than for the apex, for the rise from 15 to 20° shortens the inhibition of the base to 0.2; whilst the apex is shortened only to 0.4 (table 3).

b. The favorable effect of increasing the temperature appears more pronounced on the anterior strips, which start in $\frac{3}{10}$ of the time, than on the posterior strips, which start in $\frac{4}{10}$ of the time, when raised from 15 to 20° (table 6).

c. The favorable effect of increasing temperature appears more pronounced on aerated than on non-aerated hearts; for when raised from 15

TABLE 6

Temperature quotient (Q_t 5°) of absolute inhibition

	$\frac{20°}{15°}$	$\frac{25°}{20°}$	$\frac{30°}{25°}$
Aerated ventricle:			
Base	0.2		
Apex	0.4		
Longitudinal, Anterior and Posterior	0.2		
Non-aerated ventricle, Longitudinal:			
Anterior and Posterior	0.4	1.1	0.3
Anterior	0.28	1.2	—
Posterior	0.4	4.0	—

to 20°, the aerated longitudinal strips start in $\frac{2}{10}$ the time, the non-aerated in $\frac{4}{10}$ the time (table 6).

The data that we have already presented show that the rhythmicity is higher in the base than in the apex; in the anterior strips than in the posterior strips; in the aerated than in the non-aerated heart. It appears therefore that the favorable effect of raising the temperature is greater with conditions that themselves favor the rhythmicity of the heart. In other words, there is a certain potentiation, rather than a mere addition, of the favorable factors.

Increase of the temperature from 20° to 25° does not lead to further shortening of the absolute inhibition; on the contrary, it prolongs this inhibition (table 5) and this unfavorable effect is much more marked with the less rhythmic posterior than with the more rhythmic anterior strips (table 6). Evidently, this temperature is beyond the optimum, an

develops some factor unfavorable to rhythmicity; perhaps a too rapid consumption of oxygen, as these experiments were made only on non-aerated hearts.

Further increase of temperature from 25° to 30°, apparently raises the rhythmicity to such a degree that it again overcomes the unfavorable factor.

To summarize: Increase of temperature from 15 to 20 shortens the duration of absolute inhibition very materially; i.e., the rhythmicity is increased. This favorable effect potentiates itself with other factors that favor rhythmicity, such as the gradient and aeration.

Further increase of temperature from 20° to 25° does not continue to shorten, but tends to prolong the absolute inhibition. Some unfavorable effect, perhaps too rapid consumption of oxygen, counteracts the expected increase of rhythmicity. This interference is more marked in less rhythmic hearts.

Between 25° and 30°, the increase of rhythmicity is so great that the absolute inhibition is again shortened, notwithstanding the heat-injury.

The influence of low concentrations of potassium on the duration of absolute inhibition: Very low doses of potassium suffice to exert a marked retarding effect on the initiation of spontaneous rhythm. We employed 0.015 per cent of KCl, i.e., about half of the concentration naturally present in turtle serum. The experiments were made with longitudinal strips in aerated solutions, and at several temperatures. The results are shown in table 7.

It will be seen especially from the quotient of the last line that potassium prolongs the inhibition; the more the higher the temperature. In other words, potassium depresses the rhythmicity; and according to the general rule developed in the preceding section, it is therefore relatively insusceptible to improvement by increase of temperature, and more susceptible to the depression of excessive heat.

The potassium phenomena could also be stated in the sense that potassium renders the heart much less susceptible to the stimulant effects of heat, and much more susceptible to the injurious effects of heat; but the conception just outlined appears broader.

At 15° the inhibitory effect of this low concentration of potassium is so slight as to be almost doubtful. The median duration is as 15:16; the average is 15:15; but two strips out of three started earlier in Na than in NaK. The observations were made on parallel strips from the same hearts.

The influence of normal concentrations of calcium on the duration of absolute inhibition: Longitudinal strips of ventricle were immersed in a solution containing NaCl 0.7 per cent and 0.025 per cent of $CaCl_2$ (anhydrous); corresponding to the normal concentration of Ca in the turtle

TABLE 7

Influence of a low concentration of potassium on the duration of absolute inhibition

Longitudinal strips of ventricle, in aerated solutions. Time in minutes between immersion and first spontaneous beat.

SOLUTION; PER CENT	15°			20°			30°			$Q_T \frac{20}{15}$	$Q_T \frac{30}{20}$
	M	R	N	M	R	N	M	R	N		
NaCl 0.7	*16*	(0–28)	3	*3*	(2–43)	7	*1*	(0–6)	4	0.19	0.33
NaCl 0.7, KCl 0.015	*15*	(5–32)	3	*5*	(3–>60)	7	*10*	(0–41)	4	0.4	1.7
Quotient $\frac{NaK}{Na}$		1			1.7			10		2	5

TABLE 8

Influence of the normal concentration of calcium on the duration of absolute inhibition

Longitudinal strips of ventricle, in aerated and in non-aerated solutions. Time in minutes between immersion and first spontaneous beat.

SOLUTION, PER CENT	15°			20°			25°			30°		
	M	R	N	M	R	N	M	R	N	M	R	N
Aerated:												
NaCl 0.7	*16*	(0–28)	3	*3*	(0–43)	11				*1*	(0–6)	4
				*5.5**	(0–8)	4						
NaCl 0.7, $CaCl_2$ 0.025	*9.5*	(6–60)	4	*5.5**	(3–9)	4				*1*	(1–4)	3
Quotient $\frac{NaCa}{Na}$		0.6			2 (1*)						1	
Non-aerated:												
NaCl 0.7	*21*	(6–∞)	19	*8*	(2–∞)	20	*9*	(3–>60)	18	*3*	(1–57)	7
NaCl 0.7, $CaCl_2$ 0.025	*17*	(2–>60)	19	*8*	(1–27)	20	*7*	(2–23)	18	*3*	(2–7)	7
Quotient $\frac{NaCa}{Na}$		0.8			1			0.8			1	

* Parallel strips from the same hearts.

TABLE 9

Calcium on temperature quotient of absolute inhibition

Compiled from data of table 7

	$\frac{20}{15}$	$\frac{25}{20}$	$\frac{30}{25}$	$\frac{30}{20}$
Aerated, Na	0.2			0.3
Aerated, Na + Ca	0.6			0.2
Non-aerated Na	0.4	1.1	0.3	0.4
Non-aerated Na + Ca	0.5	0.9	0.4	0.4

serum. The duration and its relation to temperature and aeration are shown in table 8, and the corresponding temperature quotients in table 9.

It is seen that calcium distinctly shortens the inhibition at 15°, and also at 25°; but not at 20° nor at 30°; in other words, calcium has little effect at the optimal temperature of 20°C., but counteracts the deleterious effects of both cold (15°) and excessive heat (25°). It is difficult at present to relate these results in a simple manner to those of the other conditions; and it appears probable that the effects of calcium on rhythmicity present qualitative peculiarities.

SUMMARY. A certain period of absolute and then of partial inhibition intervenes between the immersion of freshly cut strips of the turtle heart, and the appearance of spontaneous beats, and eventually of the regular maximal rhythm. The length of both periods of inhibition may be conceived as inverse to the rhythmic tendency of the heart. They are therefore found to vary in duration according to the region from which the strip was taken, and according to other conditions.

The regional gradient is very marked, the duration of the inhibition increasing progressively in the order characteristic for cardiac rhythmicity; i.e., from

Right sinus region
Left sinus region
Base of the ventricle
Center of the ventricle
Left auricle (sinus free)
Apex of the ventricle
Right auricle (sinus free)

Longitudinal strips of the ventricle have the same inhibition as their most rhythmic region, i.e,, as the base of the ventricle. The right anterior quarter of the ventricle, which contains the more rhythmic tissue of the aortic bulb, is less inhibited than the other quarters of the ventricle.

Free supply of oxygen shortens the inhibition.

Increase of temperature also shortens the inhibition, although temperatures above 20°C. show at the same time some evidence of heat injury.

Potassium ions, even in one-half of the concentration of the blood serum, lengthen the inhibitions. Combination of several favorable conditions does not merely add, but rather potentiates the benefits; for instance, aeration or warming has a more favorable effect on the more rhythmic base than on the less rhythmic apex; warming has a more favorable effect on an aerated strip than on one that is not aerated. Conversely, the exaggerated inhibition of potassium is less benefited by aeration or by warming, than are the strips in potassium-free solutions.

Calcium chloride in the concentration of the turtle serum appears to have a more complicated effect; it does not alter the duration of absolute inhibition at the optimal temperature of 20°; but counteracts the inhibitory tendency both of low and of high temperatures (15° and 25°).

CONCLUSIONS

The initial inhibition of cardiac strips of turtle, may be considered as a reciprocal index of their rhythmical tendency. This agrees with the usually accepted cardiac gradient. It is increased by aeration and by temperature; and a combination of these produces a potentiated effect, and not a mere addition. Potassium even in low concentrations, acts in the contrary direction. Calcium has a more complicated effect, which for the present may be expressed as antagonistic to excessively low and excessively high temperature.

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THE AUTONOMIC RHYTHM OF THE TURTLE HEART STRIPS AS INFLUENCED BY THE REGIONAL GRADIENT AND VARIOUS CONDITIONS

II. The Maximal Tempo, the Average Rate and the Index of Inhibition

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The first paper of this series showed that the initial inhibition of cardiac strips from the turtle is an inverse index of its rhythmic tendency, and therefore varies with the accepted regional gradient; that the inhibition is shortened by agencies that increase rhythmicity, such as oxygen and moderate warming, and that these potentiate each other; and that the inhibition is lengthened by agencies that decrease rhythmicity, such as cold or oxygen deficiency or potassium; and that these also potentiate each other. Calcium had little effect under optimal conditions, but antagonized the injurious effects of cold and of excessive heat.

The contraction-rate or tempo of the strips also furnishes an index of their rhythmicity. This criterion is complicated by the frequent presence of irregularities, which are due chiefly to inhibition. It is therefore necessary to distinguish between the *average rate* of the heart, i.e., the actual number of beats per minute, including the inhibitions; and the *true tempo* of the heart, i.e., the maximal rate in the absence of inhibition or in the intervals between inhibition. The difference is that between the natural rate of a pendulum (the "true tempo"), and the rate obtained by interrupting the natural beat from time to time, which may be made to give a variety of "average rates." The true tempo of the heart is therefore shown by the shortest interval between two full beats, no matter how small the number of such beats in a group, or how sparsely the groups are scattered. For convenience, this tempo is expressed as a minute rate. It is remarkably uniform for similar conditions, whilst the average rate may vary greatly. This uniformity of the true tempo serves to reveal pseudo-tempos, due to regularly spaced inhibitions analogous to heart-blocks, but which give a rate much slower than the true tempo characteristic for the region and the conditions.

THE MAXIMAL TRUE TEMPO. *Regional gradient:* Table 1 shows that this follows the usual order; i.e., the true or intrinsic tempo decreases in the order: Right sinus, left sinus, ventricle base and ventricle apex; the most rhythmic region (right sinus) being 1.9 times as fast as the least rhythmic region (the apex) at 20°, and 2.4 times as fast at 15°. The tempo of the sinus free auricles is somewhat faster than would be expected; this is

TABLE 1

Regional gradient of maximal tempo, and effect of temperature

Cardiac strips of turtle in 0.7 per cent NaCl, aerated. Maximal heart rate at any time, expressed as beats per minute.

	20° C.			15° C.			$Q_T \frac{20°}{15°}$
	Median	Range	Number of experiments	Median	Range	Number of experiments	
Sinus-auricle Right	*36.0*	(36–39)	4	*22.0*	(19–23)	4	1.6
Sinus-auricle Left	*32.0*	(23–36)	4	*19.5*	(13–21)	4	1.6
Ventricle, Base	*22.5*	(20–23)	4	*13.0*	(11–15)	4	1.7
Ventricle, Longitudinal	*19.5*	(15–25)	8	*11.0*	(10–13)	5	1.7
Ventricle, Apex	*19.0*	(14–20)	3	*9.0*	(6–10)	4	2.1
Auricle, sinus-free Left	*25.0*	(18–27)	4				
Auricle, sinus-free Right	*20.0*	(18–20)	4				

TABLE 2

Aeration on maximal tempo

Longitudinal strips of ventricle in 0.7 per cent NaCl at 20°C. Maximal heart rate, expressed as beats per minute.

	AERATED			NON-AERATED		
	M*	R	N	M	R	N
Sinus-auricle, Right	*36.0*	(36–39)	4	*35.0*	(27–38)	3
Ventricle, Base	*22.5*	(20–23)	4	*21.5*	(21–26)	4
Ventricle, Apex	*19.0*	(14–20)	3	*21.5*	(17–22)	4

*In this and the following tables, "M" = median; "R" = range, "N" = number of experiments.

explained by the fact that the tone-waves in this region lead to very long pauses between the rhythmic beats; and these long rests have a favorable effect on the rhythmicity.

Oxygen deficiency: Table 2 shows that this has little if any effect on the initial maximal tempo.

Temperature: Tables 1 and 3 show that warming increases the tempo; the temperature quotient increasing with the usual regional gradient from

the right sinus to the apex. This would follow from the general law that a given stimulus produces more acceleration of a process going at slow speed, than of a process that is going at high speed. This makes it impossible to assert, with our present data, whether there is a more specific regional relation. According to the same law, the temperature quotient for 0.7 per cent NaCl is lower between 30° and 20° than between 20° and 15° (table 3).

Potassium: The concentration of 0.015 per cent of KCl, i.e., half of the serum concentration, decreases the tempo to about one-half (table 3). The temperature quotient is distinctly lower than would be expected from the slow tempo; so that potassium counteracts the beneficial effect of temperature.

TABLE 3

Temperature and cathions on maximal tempo

Longitudinal strips of ventricle in aerated solutions. Maximal heart rate, expressed as beats per minute.

	15° C.			20° C.			30° C.			$Q_T \frac{20}{15}$	$Q_T \frac{30}{20}$
	M	R	N	M	R	N	M	R	N		
NaCl, 0.7 per cent	*11*	(10–13)	5	*19.5*	(15–25)	8	*27.5*	(23–31)	4	1.7	1.4
NaCl, 0.7; KCl, 0.015 per cent	*6.5*	(5–8)	2	*9.5*	(9–20)	4				1.5	
Quotient, $\frac{NaK}{Na}$	0.6			0.5							
NaCl, 0.7; $CaCl_2$, 0.025 per cent	*15*	(13–16)	3	*22*	(21–26)	4	*41.5*	(34–44)	4	1.5	1.9
Quotient, $\frac{NaCa}{Na}$	1.4			1.1			1.5				

Calcium: The serum concentration, 0.025 per cent of $CaCl_2$, materially accelerates the tempo, by 10 to 50 per cent (table 3). The temperature quotient is as high or higher than with NaCl, notwithstanding the faster rate; so that calcium distinctly potentiates the beneficial effect of temperature. Just as with the inhibition period, the beneficial effects of calcium are least at 20°C., and increase with lower and with higher temperatures.

THE TIME-DECADENCE OF THE MAXIMAL TEMPO. The maximal tempo is not assumed at once; and after it has been attained, it slows more or less gradually with time. This may be determined by counting the beats on the tracings, and plotting the data in the form of time-curves. The "average rate," i.e., the actual number of beats per unit of time was also counted and plotted as curves. The difference between these two curves represents

the degree of inhibition during these periods. It would be wasteful to present the numerous data in full, either as tables or as curves. For the maximal tempo this would be especially superfluous since the decadence practically always follows very closely two simple logarithmic (inverted "compound interest") curves, as shown by the two heavy lines in figure 1.

The medians of each series were started on these curves at the point where the highest tempo of the series coincided with the curve, and drawing from there to the right. For instance, a series that reached a maximum

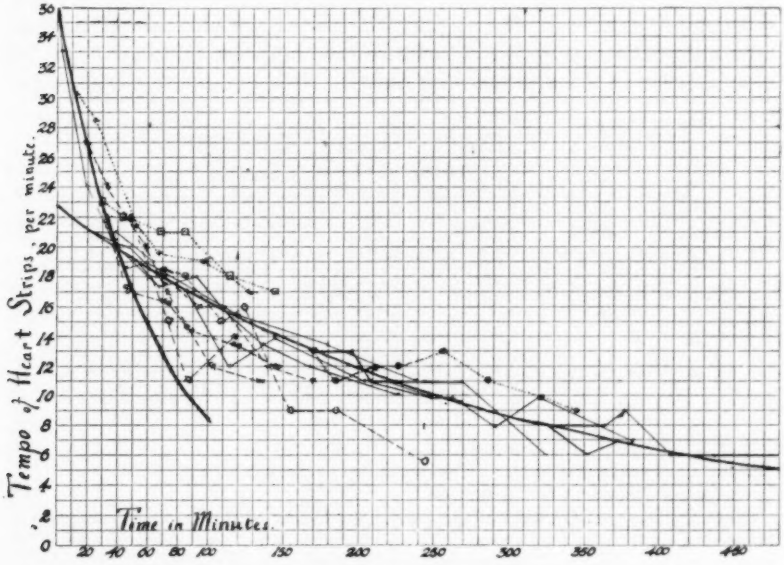

Fig. 1. The time-decadence of the maximal tempo. The experiments were divided into groups, according to regions, temperature, aeration, and cathions, as shown in table 4. The time curve of each group was then drawn, beginning with the point where the maximal tempo coincided with the two "compound interest" curves represented by heavy lines in the figure. They correspond to a striking degree, with a few rather insignificant exceptions, which are discussed in the text.

tempo of 14 beats would be started at the point where the 14th horizontal meets the curve, i.e., vertical to "150 minutes;" and if it continued to beat 120 minutes, the end-point would come at 150 + 120 = 270 minutes. The tempos prior to the maximum (0 to 20 minutes) were not plotted, since the object of the curve was confined to the decadence and not to the development of the tempo.

Figure 1 shows that the tempos fall according to the steep curve until they have reached the rate of approximately 20 beats per minute, when

they go over into the lower curve. The transition zone lies generally between 23 and 16 beats. This applies to very diverse conditions, with a few and probably insignificant exceptions to be mentioned presently. In other words, the fall of tempo during any period, say of 10 minutes, depends almost wholly on the tempo at the start of this period, and is practically independent of other conditions. The decrement, per 10 minutes, is 13.7 per cent for the steep curve, and 3.2 per cent for the flat curve, according to the calculations kindly furnished to us by Dr. G. E. Harmon. The form of the curves indicates that the decrement is due to some factor that is developed as a result, and in proportion to the rate of contraction; probably either the exhaustion of some reserve material (glycogen?) or the accumulation of a waste material (acid?). It is not due to exhaustion of

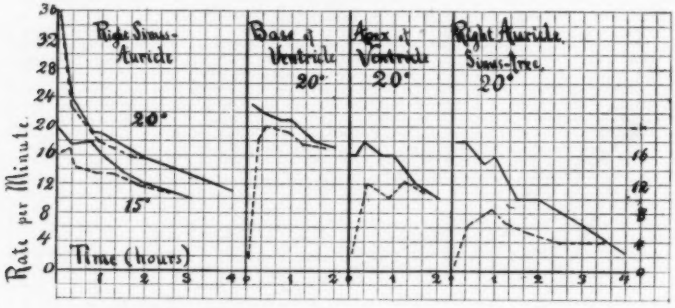

Fig. 2. The inhibition gradient.

Solid lines—maximal tempo. Broken lines—average rate.

Strips in 0.7 per cent NaCl, aerated.

Note the regional gradient of the decreasing height of the maximal rate; and of increasing inhibition, i.e., the area between the maximal and average rate. The first curve shows that cold also lowers the rate and increases the area of inhibition.

oxygen, since there is no significant difference between the curve of aerated and non-aerated strips. The more rapid decrement of the higher rates suggests that an additional factor enters into their decadence.

The only hearts with tempo sufficiently high to follow the steep curve are the sinus-auricle preparations at 20°C. in 0.7 per cent NaCl, aerated and non-aerated. These turn to the flatter curve at the tempo of about 20, as has been explained. All other preparations belong to the flat-curve type, practically throughout their course.

Table 4 gives abbreviated data on the decadence of the maximal tempo, and the solid lines in figure 2 illustrate some of these data, and bring out the regional gradient of the maximal rate very strikingly.

Regional gradient at 20°C.: Table 4 and figure 2 show that the regional gradient preserves its usual order throughout the whole course of the

tempo decadence. The very few exceptions are only temporary and probably accidental and insignificant.

Temperature: Cooling to 15° slows the decadence in correspondence with the slowing of the maximal tempo. This is shown in table 4 and illustrated in the first part of figure 2. It will also be noted from the table that the cooling delays the time when the maximal tempo is attained.

Non-aeration: Table 4 shows that the decadence of the ventricular base and apex occurs with the same rate as in aerated hearts. The rapidly

TABLE 4*

Time-decadence of maximal tempo†

	20° C.				15° C.			
Time‡	0–5	5–20	60–90	120–180	0–5	5–20	60–90	120–180
A: Regional Gradient: 0.7 per cent NaCl, aerated								
Sinus-auricle, Right	36.0	36.0	19.0	15.5	19.5	19.0	16.0	12.0
Sinus-auricle, Left	25.0	30.5	19.5	17.0	0	8.0	17.0	14.0
Ventricle, Base	0	23.0	21.0	—	0	9.0	11.0	11.0
Ventricle, Apex	16.0	16.0	16.0	11.0	4.5	8.0	9.0	6.0
Auricle, sinus-free, Left	12.0	22.0	11.0	17.0				
Auricle, sinus-free, Right	0	18.0	16.0	9.0				
B: Non-aeration: 0.7 per cent NaCl								
Sinus-auricle, Right	24.0	27.0	17.0	11.0				
Ventricle, Base	9.0	21.0	19.0	—				
Ventricle, Apex	6.9	20.1	17.0	—				
C: Cathions: Longitudinal strips of ventricle, aerated								
NaCl 0.7 per cent	10.5	20.5	17.0	—	0	10.0	10.0	10.0
NaCl + KCl 0.015 per cent	4.0	6.0	7.0					
NaCl + $CaCl_2$ 0.025 per cent	10.0	22.0	14.5	12.0	0	13.0	12.0	11.0

* This table has been condensed from the original by giving only the median values, and by omitting the periods between 20 and 60 minutes, between 90 and 120 minutes, and beyond 180 minutes.

† Maximal tempo = maximal rate at any time during the period, calculated per minute.

‡ Time = minutes elapsed since first spontaneous beat.

beating sinus-auricle also does not depart materially from the standard curve, as may be seen in figure 1, *xx*——*xx*. The decadence is therefore not due to oxygen deficiency.

Potassium: This slows the initial maximal tempo so greatly, that decadence would be almost imperceptible; on the contrary, the tempo continues to increase till the heart is arrested. This is attributable to the diminished excursions.

Calcium: This also does not materially alter the decadence curve. The 20° curve is shown by *00——00* in figure 1.

THE RELATION OF THE MAXIMAL TEMPO TO THE AVERAGE RATE OF THE HEART-STRIPS, AS INDEX OF INHIBITION. When inhibition phenomena occur, the maximal rate is more or less interrupted by pauses, and the actual number of beats per minute is correspondingly smaller than the maximal rate. This difference varies inversely as the rhythmicity, and is therefore especially marked in the early periods after excision; and in the less

TABLE 5

Average rate*

	20° C.				15° C.			
Time†	0–5	5–20	60–90	120–180	0–5	5–20	60–90	120–180
A: Regional Gradient: 0.7 per cent NaCl, aerated								
Sinus-auricle, Right	35.4	29.6	16.0	14.3	16.5	16.9	13.4	11.8
Sinus-auricle, Left	24.2	23.9	17.8	16.0	3.2	4.2	12.7	13.0
Ventricle, Base	1.8	18.0	17.4	—	0.6	2.5	10.3	9.8
Ventricle, Apex	2.4	7.9	12.4	10.7	1.6	2.0	5.1	5.9
Auricle, sinus-free, Left	2.2	4.4	8.2	6.0				
Auricle, sinus-free, Right	0.8	4.3	6.8	4.0				
B: Non-Aeration: 0.7 per cent NaCl								
Sinus-auricle, Right	11.0	25.0	10.0					
Ventricle, Base	6.9	20.1	17.0					
Ventricle, Apex	0.9	14.5	16.0					
C: Cathions: Longitudinal strips of ventricle, aerated								
NaCl 0.7 per cent	20.2	19.8	15.7	—	1.8	7.0	8.5	5.0
NaCl 0.7 per cent + KCl 0.015 per cent	4.0	5.2	7.0	—				
NaCl 0.7 per cent + $CaCl_2$, 0.025 per cent	8.1	15.3	12.1	10.7	4.0	6.6	12.5	10.2

* Actual rate of contractions per minute, during the period. The data are condensed as in table 4.

† Time = minutes elapsed since first spontaneous beat.

rhythmic regions, especially the auricles; and it is exaggerated by cold. Because of the predominance of inhibition in the early periods, the time-curve of the average rates (table 5) starts below that of the maximal rate, and rises until the two time-curves merge into each other, as illustrated in figure 2. This figure shows very beautifully the progressive regional gradient of this "inhibitory area," i.e., the area between the maximal and the average rate. This area can be conveniently estimated by drawing the curves on uniform paper and cutting out and weighing the figure

described by the divergence of the two rate-curves, for each hour. The inhibition should be expressed in reference to the level of the maximal tempo; for the inhibition of alternate beats would reduce a maximal tempo of 30 to 15; a tempo of 10 to 5. The difference between the rate would appear three times as much in the first case, although the degree of inhibition is exactly the same, i.e., alternate beats. The weight of the inhibitory area is therefore reduced to a unit maximal rate of 100, by dividing by the maximal rate of the period and multiplying by 100. The results are shown

TABLE 6

*Index of inhibition**

	20° C			15°		
Time†	0–60	60–120	120–180	0–60	60–120	120–180
A: Regional gradient: 0.7 per cent NaCl aerated						
Sinus-auricle, Right	1.9	5.3	0	26.0	10.0	0.8
Sinus-auricle, Left	12.0	13.0	—	48.0	13.0	0.7
Ventricle, Base	29.0	9.5	—	56.0	9.1	3.6
Ventricle, Apex	66.0	14.0	—	83.0	27.0	0
Auricle, sinus-free, Left	91.0	60.0	60.0			
Auricle, sinus-free, Right	100.0	44.0	57.0			
B: Non-Aeration: 0.7 per cent NaCl						
Sinus-auricle, Right	24.0	11.0				
Ventricle, Base	17.0					
Ventricle, Apex	36.0					
C: Cathions: Longitudinal strips of ventricle, aerated						
NaCl 0.7 per cent	5.8			36.0	24.0	64.0
NaCl 0.7 per cent + KCl 0.015 per cent.	11.0	4.3				
NaCl, 0.7 per cent + $CaCl_2$ 0.025 per cent.	23.0	21.0		25.0	14.0	

* Calculated by plotting the time curves of maximal tempo and of average rate on uniform paper, and cutting out and weighing the area of divergence. The weight of the paper is divided by the maximal tempo, so that the units, whilst arbitrary are strictly comparable, i.e., the double of a number corresponds in fact to the doubling of the inhibition.

†Time = minutes elapsed since first spontaneous beat.

in table 6. The most consistent and significant figures are those of the first hour; for after this time the inhibition is sometimes modified by the presence or absence of fatigue.

The regional gradient of rate-inhibition: Figure 2 shows at a glance how the area between the solid and broken lines, which represents inhibition, increases in the order of sinus, ventricle base, ventricle apex, and sinus-free auricle; and how in all these cases the inhibition is greatest at the start

and diminishes with time, till the two curves merge. Table 6 gives more comprehensive data. The total inhibition during the first hour follows strictly the entire regional gradient; after the first hour there are a few insignificant shifts.

Cold: The inhibition during the first hour is much greater at 15° than at 20° (fig. 2 and table 6). After this, there are again irregularities.

Non-aeration: This provoked inhibition in the right sinus-auricle (24 units), which was practically uninhibited when aerated (1.9 units). In the base and apex of the ventricle non-aeration apparently decreases inhibition. This, however, is a mere fatigue effect; the contractions of the non-aerated strips of the ventricle rapidly become so much weakened that they are less subject to inhibition.

Potassium: The concentration of 0.015 per cent increases inhibition distinctly.

Calcium: This (0.025 per cent) presents the usual difference between 20° and 15°. It increases inhibition at 20° and diminishes inhibition at 15°.

SUMMARY AND CONCLUSIONS

The maximal tempo of sections of the turtle heart reflects faithfully the usual regional gradient. It is accelerated by heat, slowed by potassium (0.015 per cent KCl) and markedly quickened by calcium (0.025 per cent $CaCl_2$). The calcium improvement is least marked at 20°, and increases with heat and cold. The temperature quotient indicates that potassium interferes with the quickening effect of heat and potentiates with the slowing effect of cold. Aeration does not materially affect the initial maximal tempo.

The maximal tempo declines with time, the decrement being determined by the rate of contraction, and therefore following closely a "compound interest" curve (or rather two such curves, which intersect at the maximal rate of 20 per minute). The decrement is therefore due to some factor developed as the result and in proportion to the rate of contraction; probably either exhaustion of reserve materials (glycogen?) or accumulation of waste products (acid?). The same curve applies to the different regions, temperatures, non-aeration, and calcium; i.e., the decadence with these depends practically solely on their effects on the initial maximal tempo. This overshadows entirely whatever more direct effect they may have on the decadence. Potassium appears to delay the decadence, but this is explained by diminished height of the contractions.

In the presence of inhibition, the sustained or average rate lies below the maximal tempo, proportional to the degree of the inhibition. The area between the time-curves of the maximal tempo and of the average rate therefore furnishes an index of inhibition. This is greatest when the

heart starts to beat, and decreases with time, until the two curves merge. The inhibition increases typically according to the usual regional gradient. It is increased by cold and by potassium, and is decreased by calcium at 15°, but is increased at 20°. The effects of non-aeration are complicated by fatigue.

THE AUTONOMIC RHYTHM OF THE TURTLE HEART STRIPS AS INFLUENCED BY THE REGIONAL GRADIENT AND VARIOUS CONDITIONS

III. The Time-Decadence of the Height of Contractions

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The two preceding papers of the series dealt with the rhythmicity of the heart, i.e., the inhibition and the tempo, as modified by the regional gradient, temperature, aeration, potassium and calcium. The contractility of the heart is reflected in the amplitude of the lever; but this cannot be compared directly in the different regions of the heart, since it would be influenced by the size of the cardiac strip and by the direction of the fibers. If, however, the maximal shortening of each strip be taken as the unit of its contractility, the changes that the contractility undergoes in time, i.e., the time-decadence, can be expressed as percentages of this maximal contraction, and in this manner all the strips can be reduced to a common and comparable basis. The results are most conveniently presented in the form of curves drawn from the median values, obtained from three or four strips for each condition.

The regional gradient: The results with aerated 0.7 per cent NaCl at 20°C. are shown in figure 1. The sinus-free auricles are not included, since the interference of tonus waves makes definite measurements of the rhythmic contractions impossible. The figure shows that the height of contraction decreases progressively with time, at first very rapidly, then more gradually; all the curves being in fair agreement with the logarithmic curve that is shown as a heavy line. The correspondence is not perfect, however, because the decadence is a resultant of several factors, for instance, the rate as well as the force of the contractions; the size of the segment, and probably other conditions. The base of the ventricle presents the closest correspondence to the type-curve. The apex curve is characterized by a slower initial fall, which is explained by the predominance of inhibition during this period. The sinus-auricles start with a relatively abrupt fall, which is probably due to their high initial average rate.

The observations may also be presented in another form, i.e., as the time required to reduce the excursions to $\frac{1}{2}$, $\frac{1}{4}$, etc. of their maximum. This is

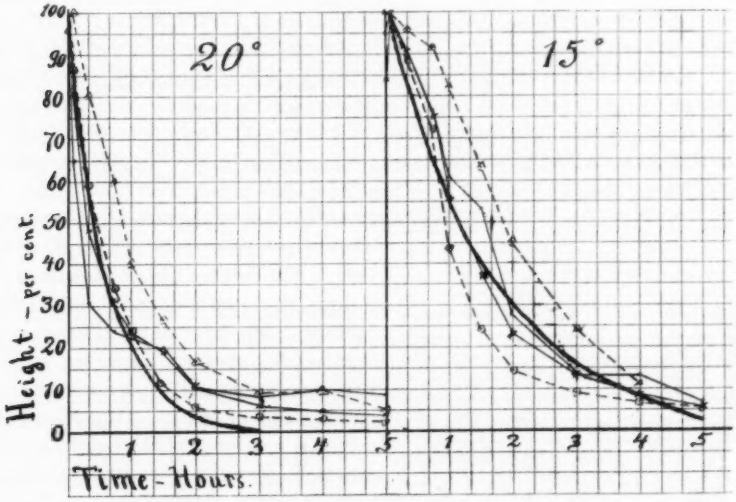

Fig. 1. The time-decadence of the height of contraction: Regional Gradient.

·———· = Sinus-auricle, right. x———x = Sinus-auricle, left. o- - - - -o = Ventricle, base. Δ- - - - - -Δ = Ventricle, apex.

The curves are the medians, each of three or four experiments. The heavy line is the logarithmic curve corresponding most closely to the general median. The strips were suspended in aerated 0.7 per cent NaCl. The time is counted from the first spontaneous beat.

TABLE 1

Time-decadence of the contraction-height of cardiac strips; regional gradient

Time required for the maximal height to fall to the following fractions (medians)

	AERATED 0.7 PER CENT NaCl AT							
	20° C.				15° C.			
Fractions	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{8}$	$\frac{1}{20}$	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{8}$	$\frac{1}{20}$
	minutes	*minutes*	*hours*	*hours*	*minutes*	*hours*	*hours*	*hours*
Sinus-auricle, Right	10	40	2	>5	70	2	3	5
Sinus-auricle, Left	19	58	2	4	75	2	3	5
Ventricle, Base	28	60	$1\frac{1}{2}$	$2\frac{1}{4}$	55	$1\frac{1}{2}$	$2\frac{1}{2}$	5
Ventricle, Apex	52	93	$2\frac{1}{2}$	5	115	3	4	5

shown in table 1 prepared from the data of the curves. The rapidity with which the contractions diminish to one-half of their maximal height is greatest in the right sinus preparations, and from this decreases through

TABLE 2

Cathions on height-decadence

Longitudinal aerated strips at 20°C.

	FRACTION			
	½	¼	⅛	1/20
	minutes	*minutes*	*hours*	*hours*
NaCl 0.7 per cent	20	50	1	1¾
NaCl 0.7 per cent + KCl 0.015 per cent.	20	40	1	1¾
NaCl 0.7 per cent + $CaCl_2$ 0.025 per cent	30	85	2½	>5

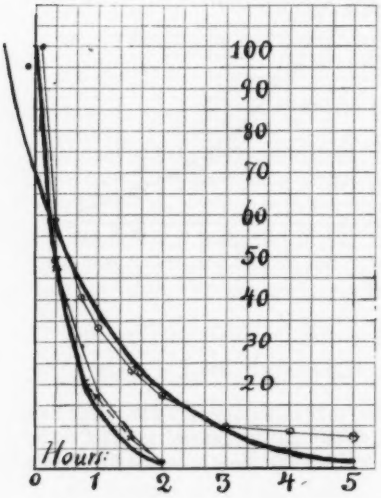

Fig. 2. Potassium and calcium on height-decadence: Longitudinal strips in aerated solutions at 20°.

·———· NaCl 0.7 per cent. x-----x NaCl 0.7 per cent + KCl 0.015 per cent. ○———○ NaCl 0.7 per cent + $CaCl_2$ 0.025 per cent.

The Na and K curves both merge almost completely in a logarithmic curve (the steeper heavy line). The calcium runs a different course and departs at both ends from the nearest logarithmic curve (the less steep heavy line).

the usual regional gradient to the apex. This might be merely the result of the rate. The further diminution does not follow the gradient, so that other factors must enter into play; for instance the greater thickness of the ventricle may lead to the gradual accumulation of acid. Altogether, the regional gradient of the height of contraction does not present any definite characteristic that might not be secondary to other factors.

The influence of cooling: Table 1 and figure 1 show that the cooling to 15° markedly retards the decadence of the height of contraction. The apex again falls most slowly; but the sinus strips show the greatest relative slowing as compared with 20°; doubtless because cooling also slows their average rate more than that of the other parts of the heart.

Potassium: Although potassium both weakens and slows the contractions, the decadence (fig. 2 and table 2) follows exactly that of sodium.

Calcium: This follows sodium and potassium closely to 20 minutes. After this time the decadence is very much slowed (fig. 2 and table 2); showing very plainly that calcium defers exhaustion. It may be recalled that calcium has very little favorable effect on rhythmicity at this temperature.

Duration of contractility: As the cardiac contractions continue, they become more feeble until they become invisible on the drum; but this occurs so gradually, that it is not possible to fix upon any definite point as the end of contractility. It is therefore better to adopt as criterion the time required to reach a definite low fraction of the maximal rate; for instance, one-twentieth. This may be gathered from tables 1 and 2. It is seen to have no definite relation to the cardiac gradient; i.e., the regional gradient is overshadowed by other factors. Cooling to 15° prolongs the endurance, but there is no material difference between the different regions. Potassium does not affect the endurance. Calcium, however, causes a very markedly longer persistence of contractions.

SUMMARY

The contractions of the segments of the excised turtle heart decrease rapidly in height, the fall following a logarithmic curve. The participation of indirect factors, such as the decadence of the heart-rate, makes it difficult to draw conclusions as to the direct influence of experimental conditions. The regional differences are not large. Cold retards the decadence. Potassium does not alter the curve. Calcium has but little immediate effect, but slows the later decadence very materially, so that its effect on contractility is much greater than its effect on the rate, at this temperature.

The endurance of the heart (i.e., the time when the contractions reach but a small fraction of the original height) does not show a marked regional gradient, nor is it materially altered by potassium. It is prolonged by cooling and by calcium.

THE AUTONOMIC RHYTHM OF THE TURTLE HEART, AS INFLUENCED BY VARIOUS CONDITIONS

IV. Types of Inhibited Rhythms and Luciani Groups

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When freshly excised strips of heart are immersed in saline solutions, they remain entirely quiescent for a time, and then start to contract, at first usually with a slow and interrupted rhythm, which after a further time attains the regular maximal tempo. The first paper of this series showed that the absolute and the partial inhibition both increase in duration with the regional gradient of the heart, from the right sinus region to the apex and thence to sinus-free auricles; and that they are lengthened by cold, by oxygen deficiency, and by potassium. The *type* of the irregularities during the period of partial inhibition also reflects the regional gradient, and the influence of the other conditions. The careful study of the curves shows that the numerous varieties of irregularities, which at first appear very confusing, may be reduced to the few types that are shown in the figures, that these develop naturally one out of the other, and are therefore an orderly index of the degree of inhibition. The course of these has been analyzed and tabulated for each tracing; but it is not necessary to reproduce these details. The irregularities practically always become progressively smaller in time, partly because the initial inhibition becomes weakened, and partly because the refractoriness diminishes with the decadence of the height of the contractions. The maximal inhibition, in each experiment, therefore is sufficient to characterize the essential course of the irregularities of that experiment.

The diminution of the irregularities from the maximal inhibition occurs often in so gradual a manner, that it is generally impossible to assign a definite time to the successive stages. The nearest approach to a time-characteristic is the period when the maximal tempo is sustained without any irregularity. This point is sometimes never reached; i.e., some degree of irregularity persist as long as the heart contracts. Where the data were adequate for conclusions, they will be included in the discussion.

The gradient of inhibition. The phenomena may be conveniently studied on strips immersed in aerated 0.7 per cent NaCl solution, at 20°

and at 15°. The sinus-free auricles are not included, since their rhythm is greatly complicated by the tonus waves.

Right sinus-auricle: At 20°, the strips started almost immediately after

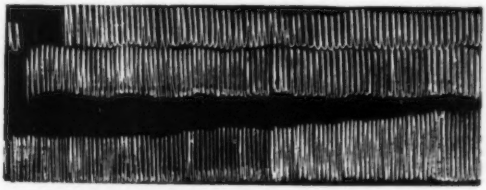

Fig. 1. *Type I:* Regular maximal tempo from the start. Right sinus-auricle strip of turtle heart, aerated 0.7 NaCl, 20°. The tracings start at the bottom, and the successive lines represent intervals of about half an hour. These and the following figures illustrate the increasingly severe types of partially suspended rhythms, and at the same time the regional gradient.

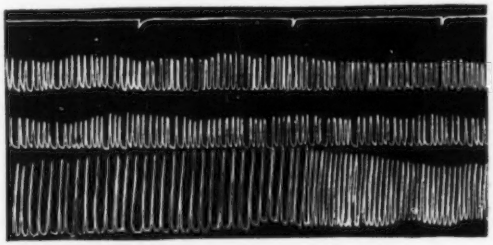

Fig. 2. *Type II:* Blocked rhythm (1:2), changing promptly into the regular maximal tempo. Left sinus-auricle, aerated 0.7 NaCl, 20°. The time signal in this and other figures represents minutes.

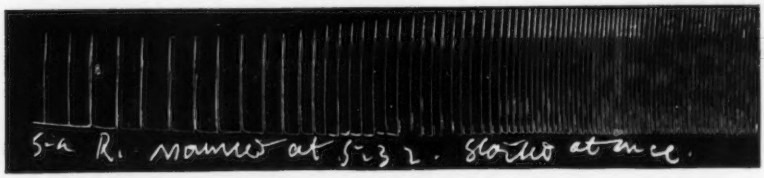

Fig. 3. *Type III:* Blocked rhythm (1:6), changing gradually but fairly promptly into the regular maximal tempo. Right sinus-auricle, *non-aerated* 0.7 NaCl, 20°. Comparison with figure 1 shows the inhibitory effect of non-aeration.

immersion, with the regular maximal tempo (type I, fig. 1). This was also the case at 15°; but very exceptionally a few scattered beats may be missed in the first few minutes, a very slight Luciani phenomenon.

Left sinus-auricle: This also started immediately after immersion, at

20° sometimes with the regular maximal tempo; but more commonly it started with a "blocked" rate, i.e., a rate of $\frac{1}{2}$ to $\frac{1}{3}$ of the maximal tempo, which after 5 to 10 minutes suddenly changed to the maximal (type II, fig. 2). The inhibition is therefore distinctly more marked than in the right sinus-auricle. At 15° the left sinus-auricle also started promptly, but the change from the blocked rate to the maximal tempo occurred gradually and much more slowly, perhaps in $\frac{3}{4}$ hour (type IV, fig. 4).

Base and middle third of ventricle: These behaved alike. At 20° spontaneous beats started in 3 minutes (median). The majority of the strips passed gradually from a blocked to the maximal tempo (types III and IV, figs. 3 and 4). The median duration of the irregularity was 13 minutes. At 15°, the inhibitory phenomena were longer of duration (absolute inhibition, median = 16 minutes; partial inhibition = 29 minutes). Their intensity varied, but averaged about the same as at 20°.

Periodic rhythms: Intermissions, resulting in "periodic rhythms" occurred exceptionally in occasional strips from the upper part of the heart; but with the apex preparations these intermissions are the rule, and present various degrees. It will therefore be advantageous to give a general description of the periodic rhythms. The lightest forms (type V, fig. 5, a and b) show the maximal tempo, regular except for occasional dropped beats. As the inhibition becomes more marked, these suspensions become more frequent and more prolonged (fig. 5b). This tracing also shows the manner in which the periodic rhythm usually develops. The first spontaneous beats generally represent a regularly blocked rhythm with a single beat between the pauses. As the inhibition becomes weaker there are two or more beats after each pause (shown also in fig. 7). If there are several beats, they occur at the maximal tempo. As the inhibition weakens further, the groups become progressively longer (the right of fig. 5b), and assume the periodic character of the Luciani rhythm. The intensity of the inhibition in the typical Luciani rhythm (type VI, lower line of fig. 6) is indicated by the relative duration of the pauses and of the rhythmic periods. The latter are always at the maximal tempo. The intermissions may be incomplete, i.e., the rhythm may be slowed or blocked instead of being entirely suspended. The upper line of figure 6 shows this change from a maximal to a half-rhythm (i.e., from 16 to 8 contractions per minute).

When the inhibition is more intense, the periodic groups become reduced to tri- or bigeminal beats (always at the maximal tempo) and finally to single beats (type VII, fig. 7). Further inhibition prolongs the pauses between these single or coupled beats (type VIII, fig. 8).

To review these processes in reverse order, the intensity of the inhibition diminishes in the following order:

1. Complete arrest of the heart.

2. Type VIII, fig. 8: *"Blocked rhythm":* i.e., occasional single or couped beats, separated by long pauses.

3. Type VII, figure 7: *"Blocked rhythm":* The pauses between the

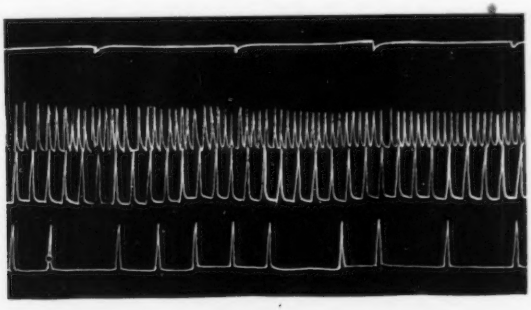

Fig. 4. *Type IV:* Blocked rhythm, changing gradually and very slowly into the regular maximal tempo. Left sinus-auricle, aerated 0.7 NaCl, 15°. Comparison with figure 2 shows the inhibiting effect of cooling.

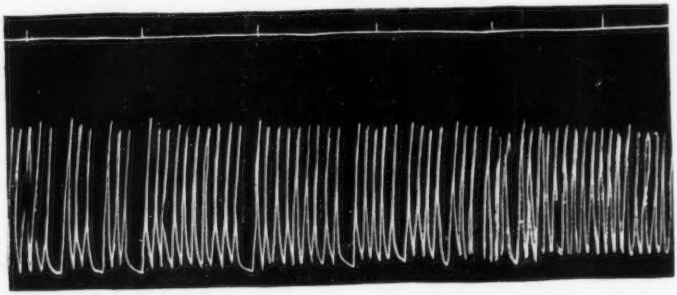

Fig. 5a. *Type V:* Periodic rhythm, light degree, occasional dropped beat Apex, 0.7 NaCl, 20°.

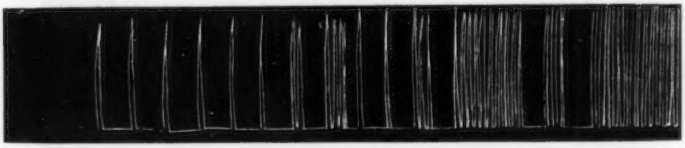

Fig. 5b. *Type V:* Development of periodic rhythm from absolute inhibition. Longitudinal strip of ventricle, 0.7 per cent NaCl, 20°.

single or coupled beats are shortened, producing a more or less regular blocked rhythm.

4. Type VI, figure 6: *"Luciani type":* groups of contractions at maximal rhythm alternating with pauses. The relative length of the periods

of rhythm and arrest indicate the intensity of the inhibition. The pauses may exceptionally be incomplete, i.e., consist of a reversion to type VII, instead of complete inhibition.

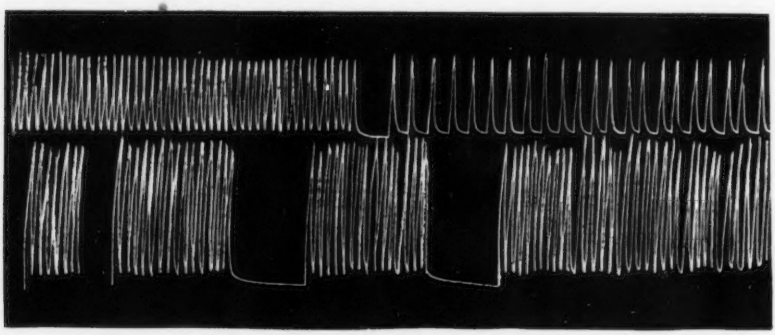

Fig. 6. *Type VI:* Lower line, periodic rhythm, fully developed. Upper line, change to blocked rhythm (1:2), instead of complete suspension. Apex, aerated 0.7 NaCl 20°.

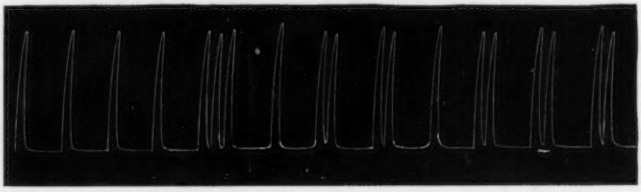

Fig. 7. *Type VII:* Blocked rhythm with relatively short pauses. Single or coupled beats. Apex, aerated 0.7 NaCl, 15°. Comparison with figure 6 shows the effect of cooling.

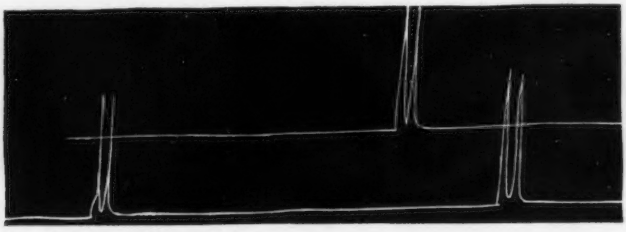

Fig. 8. *Type VIII:* Blocked rhythm (coupled beats) with long pauses. Longitudinal strips in aerated Na-K, 15°.

5. Type V, figure 5: "*Dropped beats*": The intermissions of the Luciani groups are shortened to occasional "dropped beats."

All apex-strips showed the primary complete inhibition; from this they may pass directly to any of the degrees of periodic rhythm; and from this they proceed in regular sequence through the successive types of lighter inhibition. The most intense inhibition is therefore near the beginning of the contractions, but it is not always placed at the very start: For the complete rest of the heart during the initial absolute inhibition has often stored a limited amount of "rhythmic energy," that causes the heart to make one or a few beats, or sometimes a group of beats at the maximal rate; but this spurt of energy may be soon expended and may be followed by a pause, after which the heart again resumes, sometimes with a lower order of rhythm.

Inhibition in the apex of the ventricle: At 20°, spontaneous contractions

TABLE 1

Regional gradient of inhibition

STRIPS OF TURTLE HEART IN AERATED 0.7 PER CENT NaCl	MEDIAN DURATION OF INHIBITION		TYPE OF MAXIMAL INHIBITION	
	Absolute	Partial	Median	Individual experiments
	minutes	*minutes*		
Sinus-auricle, right, 20°	0	0	I	I, I, I, I, I
Sinus-auricle, right, 15°	0	0	I	I, I, I, V
Sinus-auricle, left, 20°	0	5	I–II	I, I, II, II, II
Sinus-auricle, left, 15°	0	44	IV	IV, IV
Base of ventricle, 20°	3	13	III–IV	III, III, IV, V
Base of ventricle, 15°	16	29	II–IV	I, II, IV, VI
Longitudinal ventricle, 20°	3	—	V	II, II, III, V, V, VI, VI, VII
Longitudinal ventricle, 15°	16	—	VII	V, VII, VII, VII, VII, VII, VIII
Apex of ventricle, 20°	21	90	VI–VII	IV, VI, VII, VII
Apex of ventricle, 15°	50	∞	VII–VIII	V, VII, VIII, VIII

started in about 20 minutes (median), after immersion. Practically all the strips showed periodic rhythm of types VI and VII (Luciani group and blocked rhythm), with the median duration of 90 minutes. At 15°, the median duration of the total inhibition was 50 minutes; the periodic rhythms varied between types IV and VIII, but the majority started with types VII and VIII, i.e., with blocked rhythm. The inhibited rhythm persisted until the beats became very much weakened.

Longitudinal strips of the ventricle: Although the longitudinal strips show the same duration of total inhibition as their most rhythmic portion, i.e., as the base, their irregularities after their rhythm has started belong more to the type of the apex. At 20°, the maximal irregularities ranged from type II to type VII, with the median about type V, i.e.,

dropped beats; at 15°, they ranged between types V and VIII, with the majority of type VII, i.e., blocked rhythm.

Summary of the regional gradient: This is illustrated in table 1 which shows the regularly progressive intensification and duration of all the inhibitory phenomena in the regular order of right sinus to the apex.

TABLE 2

The influence of temperature and of cathions on the inhibitory phenomena

Longitudinal strips of ventricle, in aerated solutions

Temperature	NaCl 0.7 per cent			NaCl 0.7 per cent, KCl 0.015 per cent			NaCl 0.7 per cent, $CaCl_2$ 0.025 per cent		
	Median absolute inhibition	Type of maximal inhibition		Median absolute inhibition	Type of maximal inhibition		Median absolute inhibition	Type of maximal inhibition	
		Median	Individual experiments		Median	Individual experiments		Median	Individual experiments
°C.	minutes			minutes			minutes		
15	16	VII	III, VII, VII, VII, VII, VIII	15	VII	V, VII, VIII	10	V–VI	V, V, VI, VII
20	3	V	II, II, III, V, V, VI, VI, VII	5	IV	III, IV, IV, V	6	VI	V, VI, VI, VI
30	1	V	II, IV, V, V, V, V, V	10	IV	II, IV, V	1	IV–V	III, IV, V, VI

TABLE 3

The influence of aeration on the arrhythmic gradient

Strips in NaCl 0.7 per cent, at 20°C.

Regional gradient	Aerated		Non-aerated	
	Type of maximal inhibition		Type of maximal inhibition	
	Median	Individual experiments	Median	Individual experiments
Sinus-auricle, right	I	I, I, I, I, I	I–III	I, III
Sinus-auricle, left	I–II	I, I, II, II, II	IV–V	IV, V, VII
Base of ventricle	III–IV	III, III, IV, V	III–V	I, III, V, VI
Longitudinal ventricle	V	II, II, III, V, V, VI, VI, VII	V	II, V, V, V, V, VI, VII
Apex of ventricle	VI–VII	IV, VI, VII, VII	V–VI	I, V, V, V, VI, VI, VII

The influence of various conditions on the inhibitory irregularities. *Temperature:* The preceding descriptions and table 1 show that lowering the temperature from 20° to 15° always increases the inhibitory phenomena of each level of the heart toward that of the next

lower level. Cooling therefore tends to increase the degree of inhibition. Warming to 30° shortens the total inhibition and tends to make the type of the irregularities less severe (table 2). The improvement of rhythm between 20° and 30° is not, however, nearly so good as that between 15° and 20°. Table 2 shows that temperature influences inhibition in a similar manner in the presence of calcium and of potassium.

Aeration: Table 3 shows that the absence of aeration materially increased the type of the irregularities for the right and left sinus; the base and longitudinal strips of the ventricle have the same type of irregularity in the non-aerated as in the aerated; and the apex of the ventricle was rather less irregular in the non-aerated than in the aerated solution.

TABLE 4

The influence of aeration, calcium and temperature on the inhibitory phenomena

Longitudinal strips of ventricle

	TEMPERATURE	AERATED			NON-AERATED		
		Median absolute inhibition	Type of maximal inhibition		Median absolute inhibition	Type of maximal inhibition	
			Median	Individual experiments		Median	Individual experiments
	°C.	*minutes*			*minutes*		
NaCl 0.7 per cent...	20	3	V	II, II, III, V, V, VI, VI, VII	8	V	II, V, V, V, VI, VII
	15	16	VII	III, VII, VII, VII, VIII	21	VI	IV, VI, VI, VII
NaCl 0.7 per cent, $CaCl_2$ 0.025 per cent.....	20	6	VI	V, VI, VI, VI	8	IV	I, II, IV, IV, V, VI
	15	10	V–VI	V, V, VI, VII	17	VI	IV, VI, VI, VII

As non-aerating provokes irregularity in the otherwise regular sinus strips, and increases the *duration* of absolute inhibition also in the ventricle (table 4), it is evidently an inhibitory agency. The apparently negative effect on the *type* of rhythm in the ventricle is due to the rapid decrease in height of contraction, which tends to diminish the refractory state. Calcium at 20° also showed a less severe type of irregularity in the non-aerated than in the aerated strips; but at 15°, when the height of contraction is better sustained, the type of irregularity became practically the same.

Potassium: The addition of 0.015 per cent of KCl increased the duration of absolute inhibition, but somewhat diminished the severity of the

rhythmic irregularities, especially in the warmer hearts (table 2). This is explained by the rapid lowering of the height of the contractions.

Calcium: $CaCl_2$ 0.025 per cent (table 2) shortened the duration of absolute inhibition and diminished the severity of the rhythmic irregularities of the cooled and of the overheated heart; but it increased the inhibitory phenomena at 20°. This confirms the observation, which has been mentioned in other connections, that calcium favors the rhythm and contractility of the cooled or warmed heart, but has little effect, or is rather deleterious, to the heart at 20°.

Calcium also produced characteristic irregularities in the height of contraction, either periodic groups with constant rate, as in figure 9, or irregular groups of acceleration with diminished excursion as in figure

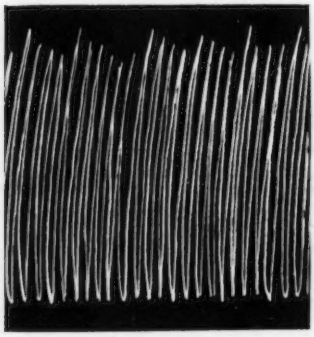

Fig. 9

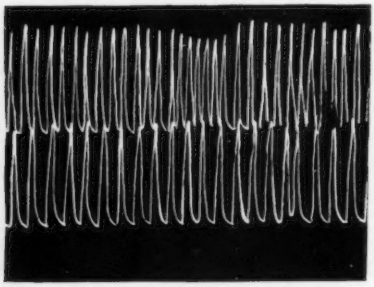

Fig. 10

Fig. 9. *Calcium irregularity:* Periodic change of height. Longitudinal strip in aerated Na-Ca, 15°.

Fig. 10. *Calcium irregularity:* Occasional acceleration with decreased height. Longitudinal strip in aerated Na-Ca 15°.

10. These phenomena occurred rarely without calcium; but in the presence of calcium they were the rule, and continued at intervals throughout the experiments.

Causation of the periodic rhythm. Periodic rhythms, identical with our types V to VIII, were described by Luciani (1) as occurring in the excised frog heart under certain conditions. He noted that the groups of contractions were longer and the pauses shorter the more of the sinus was included in the preparation. This conforms to our observations on the regional gradient. The periodic rhythm has been attributed to asphyxia and to atrio-ventricular block (Schaefer). Our observations show that asphyxia may help to provoke the intermissions; but they confirm the conclusion of Harries, that asphyxia is not the essential

element; for not only do the inhibitions occur (and are sometimes even more pronounced) in aerated solutions; but they tend to pass off in time, whereas any asphyxia should become more intense with time. As periodic rhythms were obtained from isolated portions of all parts of the heart, they are evidently not due to atrio-ventricular block. That blocked transmission within the strip may perhaps play a part, is suggested by the phenomenon shown in figure 11, obtained from an apex strip in aerated 0.7 per cent NaCl. This started 20 minutes after immersion with very feeble contractions, similar to those of the figure, and of a regular but somewhat slower temperature. These increased gradually in tempo, but not in height. In an hour after immersion appeared the first strong contraction, and from this time the strong contractions became increasingly more frequent, as shown in figure 11, until at the end of about 2 hours

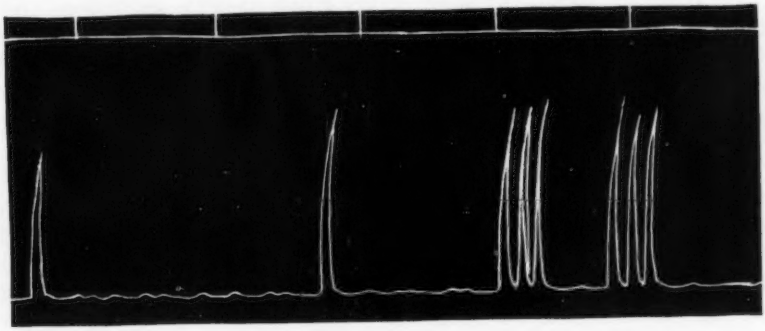

Fig. 11. *Two-phase contractions:* Minute contractions during the inhibition; observed only in this experiment. Apex, 0.7 NaCl 15°.

all the contractions were of the maximal height, preserving the tempo of the grouped high contractions in the figure. The low contractions could not have been due to deficient contractility, for such strips give a maximal contraction when electrically stimulated. It would appear, therefore, that the rhythmic stimulation that is shown by the small contractions failed to spread to the other fibers of the stip. These minute contractions during the apparent pauses were only recorded in this one experiment. It is conceivable that in other preparations the rhythmic point may have been so circumscribed as not to move the lever. It is, however, at least equally probable that this experiment was really exceptional in nature and not merely in degree; i.e., that for some reason a small part of the apex strip escaped from the inhibition which still grasped the major portion of the strip.

SUMMARY

Strips of cardiac muscle from the turtle are subject to inhibitory phenomena, consisting in longer or shorter suspension of the rhythm, with little or no direct effect on the height or on the tempo of the contractions. All the inhibitory phenomena are simply successive grades of this suspension, and follow each other in regular sequence. They are most intense immediately after the excision of the heart, when the inhibition is usually absolute (except in the sinus region). When the spontaneous beats resume, they start with a partly suspended rhythm. The degree of this initial partial suspension varies in the same direction as the duration of the initial absolute suspension. This partial suspension then diminishes in intensity, through the successive series of types, toward the regular rhythm. The time required to reach this also varies in the same direction as the duration of the initial absolute inhibition. All the inhibitory phenomena (the duration of the initial inhibition, the duration of the partial inhibition, and the type of the partially suspended rhythms) are therefore parallel. The latter, however, is somewhat complicated by the height of the contractions, since lowering of the contractions tends to diminish partial suspensions.

All the inhibitory phenomena increase with the regional gradient of the heart, from the right sinus to the left sinus, to the base of the ventricle, to the apex of the ventricle. All are increased by cold and diminished by heat. Oxygen deficiency and potassium (0.015 per cent of KCl) increase inhibition; but with these, the rapid decrease in the height of the contractions may decrease the irregularities. Calcium (0.025 per cent of $CaCl_2$) decreases the inhibitory phenomena in the cooled or overheated strips, but does not have much beneficial effect at the optimal temperature (20°).

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SECRETIN

IX. Its Relation to the Activity of Skeletal Muscle

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In a previous paper (Eddy, 1924) it was reported that a secretin preparation, perfused through the gastrocnemius muscle of the frog, increased the work performed by the muscle and delayed the development of fatigue. Since that time we have performed more than one hundred experiments dealing with the mode of action of secretin in producing this effect, particularly in reference to the following questions: Is the effect quantitative? What is the relation of the reaction of the secretin solution to its effect? What part, if any, do the vasodilator principle and other known ingredients of the secretin preparation play in the effect upon muscle? Does the active ingredient disappear from the solution during the perfusion? What is the effect of the simultaneous perfusion of glucose and secretin? Is the effect of secretin exerted upon the muscle cells or the nerve endings?

Our method of procedure was adapted from one employed by Scarborough (1921) and has been described in detail (Eddy, 1924). Briefly it consisted in recording the exhaustion curve of each gastrocnemius muscle of a frog, uniformly loaded and uniformly stimulated,—the one during perfusion of the muscle through the general circulation with Ringer's solution only, the other during perfusion in like manner with Ringer's solution plus the secretin preparation in definite amount. Brain and spinal cord of the frog were pithed. The perfusion was carried out at room temperature under constant pressure of 30 cm. of water by way of a cannula inserted into the bulbus arteriosus through an incision in the ventricle. The load for each muscle was 20 grams. Stimuli were single break shocks (make shocks short-circuited) from the secondary circuit of a du Bois-Reymond induction coil, sent in at intervals of five seconds. The contractions were recorded on a very slow drum.

When perfusion of the preparation with Ringer's solution only had been established the height of the response of one gastrocnemius muscle to a single maximal break shock was recorded. The drum was started and the contractions of this muscle to this strength stimulus were recorded to the

point of complete exhaustion. The time which this required and the total fluid perfused during this time were noted. Then the other muscle was attached to the myograph and its response to the same strength shock determined. If this was like the initial response of the first muscle, perfusion was begun with Ringer's solution plus the secretin preparation.

TABLE 1

PREPARATION AND PROCEDURE	AMOUNT PER 100 CC.	NUMBER OF OBSERVATIONS	PERFUSION RATE PER MINUTE		FATIGUE TIME		WORK DONE	
			A	B	A	B	A	B
	mgm.		*cc.*	*cc.*	*minutes*	*minutes*	*gram-mm.*	*gram-mm.*
Secretin—Stepp. Unneutralized (previous report)	10	6	5.9	7.8	26.8	33.0	15,610	18,164
Secretin—Stepp. Neutralized	10	9	6.5	8.3	22.6	26.8	12,190	13,932
Secretin—Fresh acid extract. Neutralized	10	8	2.6	3.1	24.0	27.6	14,054	15,904
Secretin—Stepp. Unneutralized	20	9	5.9	8.0	29.0	28.8	16,688	16,398
Secretin—Stepp. Neutralized	20	5	4.0	5.8	23.6	24.8	15,196	15,680
Secretin—Stepp. Neutralized	50	6	7.2	9.6	26.5	27.3	12,772	15,864
Ringer only. Reperfused		5	6.7	6.2	31.6	31.8	17,756	18,076
Secretin—Stepp. Neutralized—Reperfused	10	6	6.4	6.7	28.3	28.3	14,860	14,342
Secretin—Stepp. Neutralized—Reperfused	20	5	7.0	6.9	26.0	28.8	16,536	18,276
Secretin—Stepp. Neutralized—Reperfused	50	5	4.6	5.6	27.6	31.2	16,836	19,444
Secretin—Stepp. Neutralized—Twice reperfused	50	5	6.5	6.0	24.8	28.4	12,368	13,800
Secretin—Stepp. Neutralized—Muscle immersed	10	10			34.2	36.5	21,330	23,307
Secretin—Fresh acid extract. Neutralized—Unecurarized frog	10	8	2.6	3.1	24.0	27.6	14,054	15,904
Secretin—Fresh acid extract. Neutralized—Curarized frog	10	8	3.1	3.6	27.0	27.6	15,806	16,412
Secretin—Fresh acid extract. Neutralized—Plus glucose	10	14	2.9	3.4	25.4	28.3	15,102	16,210
Histamine	1	7	2.9	2.4	21.7	21.5	10,746	10,486

A, during perfusion with Ringer's solution only.

B, during perfusion with Ringer's solution plus the preparation indicated (column 1).

At the end of a minute the record of the second exhaustion curve was started. Again fatigue time and total amount of fluid perfused were noted. Subsequently the work performed by each muscle was calculated from the tracings. Our results are summarized in tables 1 and 2.

Our secretin preparation, a dried acid extract (Downs and Eddy, 1917),

purified according to the method of Stepp (1912), in the proportion of 10 mgm. per 100 cc. of Ringer's solution, increased the work done 16.33 per cent and lengthened fatigue time 23.17 per cent. Twenty milligrams of the same preparation per 100 cc. of Ringer's solution had practically no effect; the average figures show a slight decrease in work done, 1.73 per

TABLE 2

Percentage differences

PREPARATION AND PROCEDURE	AMOUNT PER 100 CC.	NUMBER OF OBSERVATIONS	PERFUSION RATE		FATIGUE TIME		WORK DONE	
			Increase	Decrease	Increase	Decrease	Increase	Decrease
	mgm.							
Secretin—Stepp. Unneutralized (previous report)	10	6	32.20		23.17		16.33	
Secretin—Stepp. Neutralized	10	9	27.69		18.58		14.29	
Secretin—Fresh acid extract. Neutralized	10	8	19.39		15.00		13.09	
Secretin—Stepp. Unneutralized	20	9	35.59			0.62		1.73
Secretin—Stepp. Neutralized	20	5	45.00		5.08		3.18	
Secretin—Stepp. Neutralized	50	6	33.33		3.01		24.20	
Ringer only. Reperfused		5		7.46		0.63		1.81
Secretin—Stepp. Neutralized—Reperfused	10	6	4.68		0.00	0.00		3.48
Secretin—Stepp. Neutralized—Reperfused	20	5		1.42	10.76		10.52	
Secretin—Stepp. Neutralized—Reperfused	50	5	21.33		13.04		15.49	
Secretin—Stepp. Neutralized—Twice reperfused	50	5		7.69	14.51		11.57	
Secretin—Stepp. Neutralized—Muscle immersed	10	10			6.72		9.23	
Secretin—Fresh acid extract. Neutralized—Unecurarized frog	10	8	19.39		15.00		13.09	
Secretin—Fresh acid extract. Neutralized—Curarized frog	10	8	17.74		2.31		4.46	
Secretin—Fresh acid extract. Neutralized—Plus glucose	10	14	17.24		11.41		7.33	
Histamine	1	7		15.98		0.92		2.41

cent, and fatigue time, 0.62 per cent. Abel (1907), Dale and Mines (1911), Grant (1920) and others have commented on the depressant effect upon skeletal muscle of fluctuations in the H-ion concentration. Since our secretin preparation was originally an acid extract, we turned our attention to the reaction of our solution for an explanation of the disappearance of effect with the larger dose.

Our Ringer's solution was composed of sodium chloride 0.65 gram, potassium chloride 0.03 gram, calcium chloride 0.026 gram, distilled water to 100 cc. and had a pH of 6.3. The secretin preparation, 10 mgm. per 100 cc., gives a solution with pH 4.5; 20 mgm. per 100 cc. gives a solution with pH 3.8. Using phenol red (phenolsulphonephthalein, 0.02 per cent), one drop per 100 cc., as indicator, we added N/100 NaOH to equal bulks of Ringer's solution and secretin solution to bring them both to pH 7.4. For convenience we shall refer hereafter to solutions treated in this way as "neutralized." Perfusion of neutralized secretin solution, 10 mgm. per 100 cc., increased the work done 14.29 per cent and increased fatigue time 18.58 per cent. This was essentially the same effect, in degree as well as kind, as was produced by the untreated solution. Reaction does not affect the result materially in perfusion with this strength solution. But perfusion of neutralized secretin solution, 20 mgm. per 100 cc., increased the work done 3.18 per cent and fatigue time 5.08 per cent. This is still only a fraction of the effect produced by the smaller dose; but it indicates that the neutralization has partially enabled the stimulating influence of the preparation to manifest itself. Perfusion of neutralized secretin solution, 50 mgm. per 100 cc., increased the work done 24.20 per cent and increased fatigue time 3.01 per cent. This is a greater effect than with 20 mgm. but hardly better than with 10 mgm. of the preparation. Evidently the H-ion concentration had depressed the muscle so as to prevent the augmenting action of the secretin in the larger dose; but it seems to us that there is present also some other depressant agent, the nature of which we have not yet been able to determine. It is not histamine. This substance has been shown to be present in secretin preparations (Barger and Dale, 1911; Parsons, 1925). It is probably present in our preparation; 10 drops of a 1 per cent solution, neutralized, added to 100 cc. of warm Ringer's solution, in which is suspended a strip cut from the cornu of the non-gravid uterus of the rabbit, have a marked oxytocic effect. Histamine in the proportion of 1 mgm. per 100 cc. of Ringer's solution, a quantity certainly in excess of what could be present in the amounts of secretin preparation which we have employed, has little or no effect upon muscle.

If the neutralized secretin solution, 10 mgm. per 100 cc., after it had passed through the vessels of the frog, were reperfused, it was found that it had lost its property of increasing the work done by muscle and had lost almost entirely its effect on perfusion rate. If neutralized secretin solution, 20 mgm. per 100 cc., were similarly reperfused, again there was a marked decrease in its influence upon perfusion rate; but the work done and fatigue time were increased more than when the solution was first perfused. This increase is not due to the addition of any agent to the perfusing fluid during its passage through the vessels, since Ringer's

solution alone reperfused has no stimulating effect upon muscle. Rather it would seem to be due to the removal in the tissues of the depressant agent referred to above, apparently removed from the solution at a faster rate than the agent which improves the working power of the muscle. Since the vasodilator agent is also diminished in a similar manner, there is a strong presumption that it and the depressant agent are the same. Similar results were obtained by perfusing neutralized secretin solution, 50 mgm. per 100 cc., and reperfusing the perfusate. During the first perfusion of this solution fatigue time was increased 3.01 per cent, the work done was increased 24.20 per cent, and perfusion rate was increased 33.33 per cent. When the solution was reperfused fatigue time was increased 13.04 per cent and the work done 15.49 per cent but the perfusion rate was increased only 21.33 per cent. When this solution was passed through the frog's vessels a third time a marked increase was still manifest in fatigue time, 14.52 per cent, and in work done, 11.57 per cent, but the perfusion rate was decreased 7.69 per cent. These results show clearly that the specific principle of the secretin preparation as well as the vasodilating agent disappear from the solution during perfusion, the former at a rate close to one milligram of the whole preparation per minute. Furthermore, we believe these results indicate again that the specific physiologically active substance in secretin preparations is independent of the common vasodilator agent of tissue extracts.

The experiments recorded so far were performed with a purified secretin preparation as noted. A fresh dried acid extract, carefully neutralized, was found to have a similar effect in kind, though slightly less in degree (see table 2). The remaining experiments of this paper were performed with this latter preparation.

We next tried the addition of glucose to the perfusion fluid. It was added to the Ringer's solution in amount approximately equal to the sugar content of the frog's blood. Then the secretin preparation was added in appropriate amount to a part of this Ringer-glucose solution and finally the pH was adjusted as described.

Using the Shaffer-Hartman (1921) method we determined the percentage of sugar in the blood of the frogs used, also the percentage of sugar in the Ringer-glucose and secretin-Ringer-glucose solutions. Then the experiment was carried out as before, exhausting one muscle during perfusion with Ringer-glucose and the other during perfusion with secretin-Ringer-glucose. Perfusion rate was noted in each case as usual and sugar determinations were made on the perfusates (see table 3).

With Ringer-glucose the sugar concentration in the perfusate fell slightly, 0.072 to 0.071 per cent, while with the secretin solution it rose, 0.072 to 0.078 per cent. Snyder, Martin and Levin (1922) and Wells (1923) drew attention to the fact that sugar output from the liver varies with the per-

fusion rate and emphasized the necessity of determining minute output of sugar as well as concentration in experiments involving any factor which might affect glycogenolysis. In our experiments perfusion was through the general circulation including the liver and perfusion rate was increased by the secretin solution, on the average 17.24 per cent. Calculating the minute output of sugar we find that with Ringer-glucose solution only it averaged 2.059 mgm. and with secretin solution it averaged 2.652 mgm., an increase of 28.80 per cent as compared with the 17.24 per cent increase

TABLE 3

EXPERIMENT NUMBER	SUGAR CONCENTRATION		PERFUSION WITH RINGER'S SOLUTION				PERFUSION WITH SECRETIN SOLUTION				PERCENTAGE INCREASE	
	Frog's blood	Perfusion fluid	Rate	Duration	Sugar concentration in perfusate	Sugar weight output per minute	Rate	Duration	Sugar concentration in perfusate	Sugar weight output per minute	Minute volume of perfusion	Minute glucose output
	per cent	*per cent*	*cc.*	*minutes*	*per cent*	*mgm.*	*cc.*	*minutes*	*per cent*	*mgm.*		
73		0.100	3.5	30.0	0.087	3.045	2.3	34.0	0.097	2.231	34.28*	26.73*
74		0.100	3.4	26.0	0.090	3.060	2.3	26.0	0.094	2.162	32.28*	29.34*
78		0.097	2.9	28.0	0.079	2.291	3.2	27.0	0.087	2.784	10.34	21.51
79		0.097	0.5	19.0	0.078	0.390	2.0	21.0	0.087	1.740	300.00	346.15
83		0.048	2.8	23.0	0.070	1.960	4.0	33.0	0.064	2.560	42.85	30.61
84		0.048	2.1	29.0	0.054	1.134	2.7	31.0	0.054	1.458	28.57	28.57
86	0.078	0.069	3.2	31.0	0.078	2.496	4.0	35.0	0.064	2.560	25.00	2.56
87	0.064	0.061	4.0	26.0	0.056	2.240	4.4	26.0	0.063	2.772	10.00	23.75
88	0.041	0.067	3.0	24.0	0.063	1.890	4.0	26.0	0.071	2.840	33.33	50.26
89	0.047	0.066	1.6	30.0	0.071	1.136	2.0	36.0	0.073	1.460	25.00	28.52
90	0.063	0.065	2.0	24.0	0.065	1.300	3.5	23.0	0.078	2.730	75.00	110.00
96	0.051	0.061	4.6	23.0	0.054	2.484	5.2	25.0	0.070	3.640	13.04	46.53
97	0.058	0.072	3.7	21.0	0.077	2.849	4.0	23.0	0.077	3.080	8.10	8.10
98	0.076	0.077	4.2	22.0	0.077	3.234	4.0	30.0	0.091	3.640	4.76*	12.55
Average..	0.061	0.072	2.9	25.4	0.071	2.059	3.4	28.3	0.078	2.652	17.24	28.80

* Decrease.

in perfusion rate. This relationship held true in all but three experiments and in two of these minute volume and minute glucose output were increased to the same extent (see table 3). Assuming an approximately direct relationship between perfusion rate and sugar output it would seem that this difference is greater than can be accounted for by the increase in perfusion rate alone. We believe secretin is a direct cause of the liberation of sugar from some source within the organism, most probably the liver. However, the observation needs to be checked again in accordance with he suggestions made by Wells (1923).

Curiously enough, in spite of this effect upon the sugar content of the perfusing fluid, we found that secretin dissolved in Ringer-glucose had less effect upon the muscle than secretin dissolved in Ringer's solution alone. In the former case fatigue time was increased 11.41 per cent and work done was increased 7.33 per cent; in the latter case fatigue time was increased 15.00 per cent and work done was increased 13.09 per cent. In both perfusion rate was affected to about the same degree, being increased 17.24 per cent and 19.39 per cent respectively.

All of these experiments had been performed on pithed frogs so that the observed effect was a peripheral one, upon muscle cell or nerve ending. It was not a function of the perfusion rate. We tried the effect of immersing the gastrocnemius muscle in a secretin solution, 10 mgm. of the preparation per 100 cc. of Ringer's solution, neutralized as before. An exhaustion curve was made in the usual manner with the muscle so immersed, and compared with the exhaustion curve of the other gastrocnemius of the same frog, immersed in Ringer's solution only. The muscle subjected to the influence of secretin did 9.23 per cent more work and its fatigue time was lengthened 6.72 per cent. This was a much smaller effect than was produced by a secretin solution of the same strength perfused through the muscle. This might be accounted for on the basis of incomplete penetration of the solution into the muscle. On the other hand, it might be because the secretin acts upon the nerve endings, which would rapidly degenerate in the isolated preparation. We proceeded to compare the effect of perfusion of the same secretin solution in curarized and uncurarized frogs.

A solution of the secretin preparation, 10 mgm. per 100 cc., was made and neutralized as described. The effect of this solution on the muscle of an uncurarized frog was determined. Another frog was curarized by injection of 1 per cent curare solution into the anterior lymph sac. The completeness of the curare paralysis was determined by stimulating the exposed sciatic nerve with single break shocks of the strength to be used in the experiment. Then the frog was prepared and perfused using another portion of the secretin solution the effect of which had just been determined on an uncurarized frog. It was found that this solution increased the work done 13.09 per cent and fatigue time 15.00 per cent, when perfused through an uncurarized muscle; but it increased the work done only 4.46 per cent and the fatigue time only 2.31 per cent, when perfused through a curarized muscle. Apparently the greater part of the action of the secretin preparation had consisted in an increase in the excitability of the motor nerve endings. We conclude that secretin had little direct influence upon the metabolism of skeletal muscle.

CONCLUSIONS

1. The fact that secretin preparations increase the working power of a muscle and delay the development of fatigue is confirmed.

2. This effect may be influenced by but is not dependent upon the reaction of the secretin solution.

3. The effect is not due to the vasodilator agent in secretin preparations.

4. It is not due to histamine.

5. It is due to an agent in the preparation which disappears from the solution during perfusion. The vasodilator substance also disappears from the solution during perfusion. The rate of disappearance is not the same for the two substances.

6. The effect is very much less in curarized frogs, indicating that the preparation has acted mainly upon the motor nerve endings.

7. The effect is decreased when glucose is also added to the perfusing fluid, although the secretin preparation causes an increased sugar output from some source within the organism.

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